FEDERAL EXPERIMENT STATION IN PUERTO RICO

of the

UNITED STATES DEPARTMENT OF AGRICULTURE

MAYAGUEZ, PUERTO RICO

REPORT OF THE FEDERAL EXPERIMENT STATION IN PUERTO RICO 1946

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FEDERAL EXPERIMENT STATION IN PUERTO RICO
MAYAGUEZ, PUERTO RICO

Administered by the Office of Experiment Stations
Agricultural Research Administration
United States Department of Agriculture

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[1] In cooperation with the Government of Puerto Rico.

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INTRODUCTION

The Federal Experiment Station in Puerto Rico was established in 1901. During the early years of its existence the station activities were concerned mainly with problems of local interest. However, in 1935 a new policy was established whereby the Federal Experiment Station was to be strengthened as a tropical research outpost of the United States Department of Agriculture, rather than to continue as an agency solely for the agricultural interests of Puerto Rico.

Since 1935, in addition to the regular Federal appropriation the station has received nearly $800,000 from miscellaneous Federal and Insular funds such as the Civilian Conservation Corps, the National Youth Administration, the Work Projects Administration, the Puerto Rico Reconstruction Administration, and the War Emergency Program. These funds were used largely for the opening up of new land, improvement of roads, and the construction of buildings. As a result of these activities the station now has a well-developed physical plant and within the past year has acquired an additional 22 acres of land through funds provided by the Insular Government, making a total of about 600 acres of land, mostly at Mayaguez, under the jurisdiction of the Department. Through cooperation with the United States Forest Service the station also maintains three substations in the Caribbean National Forest areas, one at Maricao at 2,300 feet elevation, one at Toro Negro at 3,300 feet elevation, and another near Guánica at sea level under arid conditions. Thus, the land facilities of the station provide a wide range of environments for the carrying on of tropical research.

Since 1938 the station has received a small annual appropriation from the Insular Government, amounting to approximately $25,000, and this appropriation continued until 1946 when it was raised to $56,780. The money appropriated by the Insular Government is not available for support of the Federal research program but represents expenditures for research projects in which the Insular Government is particularly interested from the standpoint of Puerto Rican agriculture. The Federal station provides facilities and supervision for the carrying out of this program. The projects include agronomic and chemical studies on vanilla, spices, essential oils, and bamboo.

In connection with one of its major activities, new plant introductions, the station has introduced more than 8,000 species of tropical plants which represent one of the largest, if not the largest, botanical collection of tropical plants in the Western Hemisphere. Within this collection are to be found such strategic crops as rubber, *Cinchona*, and *Derris*. Research on the latter two crops now in progress has for its objective to improve and maintain these crops and to insure the United States a source of high-quality propagation material in the event the crops are needed in the future as they were during World War II.

The station has cooperated closely with other bureaus of the United States Department of Agriculture in the distribution of plant material and insect parasites and predators, and in increasing continental seed of new and better varieties during the winter season. These projects are only a few of those in which the station has been able to make direct contributions to the development of agricultural research of interest and importance to the continental United States.

In recent years the development of the "Good Neighbor Policy" and the increased facilities for travel between North and South America have brought the station closer to its Latin American neighbors, a majority of whom reside under tropical conditions. Already, the United States is helping these nations by the establishment of agricultural experiment stations within their own borders. Puerto Rico, geographically located between North and South America, offers an excellent meeting place for programs sponsoring the betterment of tropical agriculture. The Federal Experiment Station over a period of 44 years has already made many noteworthy contributions to the Latin American program. A single and recent example was the distribution of over 2,000,000 cuttings of *Derris elliptica* for the purpose of increasing war production of the strategic insecticide, rotenone.

Now, more than ever before, the United States Department of Agriculture has need for research in tropical agriculture. World affairs have carried us willingly or unwillingly far beyond the shores of the United States mainland, and we are faced with problems in many parts of the world in which scientific knowledge of tropical agriculture is needed.

The following pages contain accounts of progress made at this station during the past year in research with tropical crops.

PERSONNEL

Several personnel changes occurred on the Federal staff during the year. Arnaud J. Loustalot, formerly plant physiologist with the USDA Field Laboratory for Tung Investigations at Gainesville, Fla., joined the staff as chemist on August 12, 1945. There were three resig-

nations. Roy E. Harper, geneticist, left the station staff on July 12 to enter private business in Texas; Barton C. Reynolds, agricultural engineer, transferred to the Office of Experiment Stations, Washington, D. C., as experiment station administrator on July 25; Milton Cobin, horticulturist, resigned on September 12 to join the administrative staff of the University of Puerto Rico, Río Piedras, P. R.

A number of changes were made during the year in the personnel employed under funds provided by the Insular Government. The following appointments were effective July 1, 1945: Aida G. Villafañe and Rubén H. Freyre, agronomists; José B. Huyke, agricultural engineer, and Iris C. Vera, clerk-stenographer. Susana Rodríguez was appointed clerk-stenographer on September 20, 1945. Mrs. Edna R. Cedó, clerk-stenographer, resigned on September 19, 1945.

During the absence of the director, Norman F. Childers and Arnaud J. Loustalot served in the capacity of acting director for periods totaling approximately 2 months.

COOPERATION WITH OTHER GOVERNMENT AGENCIES

Excellent cooperation was received during the year from the Insular Government. An increased appropriation amounting to $56,780.00 was made available to the Federal station to carry on cooperative experimental work with specialty crops for Puerto Rico, including vanilla, spices, essential oils, and bamboo.

Through an appropriation of $23,000 from the Insular Legislature, approximately 22 acres of land were added to the station property to be used for new plant introductions, an experimental mango orchard, and a vegetable trial garden.

The experiment station of the University of Puerto Rico and the Federal station continued close cooperation on agricultural problems. Through conferences and exchange of programs the respective directors coordinated all activities in order to avoid duplication of projects. The Federal station continued to provide office, laboratory, and field space for the experimental coffee work at Mayaguez conducted under the direction of the Insular station.

The Institute of Tropical Agriculture of the University of Puerto Rico, located at Mayaguez, has cooperated with the station in the exchange of plant material, library facilities, and use of equipment. The director of the station served as a member of the board of trustees of the institute.

The College of Agriculture and Mechanic Arts of the University of Puerto Rico located at Mayaguez frequently sent classes in horticulture to the station to examine the experimental projects on vegetables, forage and pasture crops, and tropical plants in general.

The extension service of the University of Puerto Rico gave excellent cooperation to the station by securing native plant material for comparison with introduced varieties. A number of experimental plantings of promising plant introductions, particularly tropical kudzu, were made on the demonstration farms of the extension service as a means of introducing these plants to farmers over the island. Considerable quantities of bamboo, USDA–34 sweet corn, and other plants were distributed through the extension service.

The Federal and Insular Forest Services worked closely with the station during the past year, particularly in a bamboo propagation and distribution program. The Insular Forest Service provided labor for propagating, digging, and transporting several thousand offsets of improved bamboo varieties to watershed plantings of the Forest Service in the mountainous areas of Puerto Rico. The Forest Service continued to make land available to the station at Toro Negro, Maricao, and Guánica for cinchona, vegetable, and plant introduction programs.

The Puerto Rico Development Co. continued cooperation with the station in preparing and distributing bamboo culms for the development of new bamboo industries on the island. A fishing rod manufacturing company, utilizing varieties of bamboos introduced by the station, was established recently near Mayaguez.

A cooperative project was initiated with the Puerto Rico Agricultural Co. for the purpose of propagating and establishing commercial orchards of leading varieties of mangos and avocados which the station has maintained in its plant collection for many years.

The United States Army at Borinquen Field and the Navy at San Juan have been exceptionally cooperative in providing personal services and materials to the station. In return, the station has made available a considerable quantity of planting material and has provided technical advice to these and other military bases in Puerto Rico.

In serving as a tropical outpost of the United States Department of Agriculture, the station cooperated with several other bureaus and agencies within the Department. A large amount of seed and planting material were supplied to the Office of Foreign Agricultural Relations for introduction and testing at their experiment stations in Latin America. The exchange of ideas and reports between staff members of both organizations was of considerable mutual benefit.

The Bureau of Entomology and Plant Quarantine, through the facilities of its South American Parasite Laboratory, made available several shipments of insect parasites and predators.

The Bureau of Plant Industry, Soils, and Agricultural Engineering through its Division of Plant Exploration and Introduction, made available a considerable quantity of seed and planting material. The station was able to supply material for reciprocal requests.

Cooperative relations were maintained with the USDA Regional Vegetable Breeding Laboratory, Charleston. S. C., in the testing of new varieties developed by the laboratory, and likewise, the station was able to increase seed material for them during the winter months.

The station received excellent cooperation from the Soil Conservation Service in cooperative projects, particularly with promising new pasture and soil erosion crops for Puerto Rico. Laboratory space, offices, and field areas were supplied by the station for experimental and observational studies by the Soil Conservation Service. Office space was also made available to the Farm Security Administration and to an Insular plant-quarantine inspector who is a collaborator of the Bureau of Entomology and Plant Quarantine of the Department.

The director of the station served as a member of the USDA War Board for Puerto Rico until the end of the war when the duties of this organization were merged with those of the USDA Council.

INSECTICIDAL-CROP INVESTIGATIONS

DISTRIBUTION

By David G. White

Cuttings of the superior MG derris clones were distributed.—The distribution of planting material of *Derris elliptica* and *Lonchocarpus* spp. was largely confined to experimental material. A total of 655 mixed MG clones Nos. 1-9 of *D. elliptica* was sent to the Estación Experimental de Cinchona of the Office of Foreign Agricultural Relations in Guatemala. Also 2,665 plants of the Sarawak Creeping variety of *D. elliptica* and 20 cuttings of *Lonchocarpus* sp. were sent to the same station.

Two small shipments of MG clones and 1,800 cuttings of the Sarawak Creeping variety of *Derris elliptica* were sent to private growers in Guatemala. In addition, small shipments of MG clones were made to Dominica and Cuba. A shipment of 5,000 cuttings of the Sarawak Creeping variety of *D. elliptica* was made to Manáos, Brazil.

PROPAGATION

By David G. White, Merriam A. Jones, and Caleb Pagan

Rate of Derris root elongation was not correlated with rotenone content.—Measurements of the rates of root elongation from derris cuttings grown in boxes with glass sides were discussed in a previous report (*31, p. 7*).[1] The relation of rotenone content to the rate of root elongation was investigated. The variation in rotenone content of roots classified as slow-growing and as fast-growing was equally as great within a group as between groups. No correlation was found between the rate of root elongation at an early age of the plant and rotenone content.

Mature Derris plants were not influenced by type of cutting planted.—The seventh harvest in this experiment on the effect of type of cutting material of derris on subsequent root development and rotenone content (*16, 30, p. 6*) was made on October 1 and 2, 1945. The plants had been in the field for 23 months and in nurseries 3 months before field planting. Variation in yields among replicates of any one treatment was so great as to preclude any outstanding difference between treatments. Therefore, the yield of roots of mature plants of derris was concluded to be similar regardless of the type of cutting used for propagation. The amount of roots produced in the last 6 months was comparatively small. By comparing these data with those taken 6 months previously it was calculated that the increase amounted to only 4.5 percent of the total weight of the roots. Although rotenone percentages, which varied from 3.4 to 7.4 percent, had increased slightly during the last 6 months, they could not be definitely correlated with treatments.

Mulching Derris resulted in lower soil temperatures.—In a previous report (*27*) the preliminary results on the reduction of soil temperatures under mulch were discussed. In table 1 the accumulated temperature data are presented for two different mulches at different depths below the soil surface. The field was planted with the Changi No. 3 variety of *Derris elliptica* during January 1945 and was originally mulched during the first week of March. A second application of mulch was made during the first week of August 1945.

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[1] Italic numbers in parenthesis refer to Literature Cited, p. 54.

TABLE 1.—*Soil temperatures beneath different mulches in a planting of the Changi No. 3 variety of Derris elliptica*

Kind of mulch	Range of soil temperature	Greatest difference[1]	Depth measured[2]	Air temperatures	Average rainfall per day	Period measured
	°*F.*	°*F.*	*Inches*	°*F.*	*Inches*	*1945*
12 inches lemon grass	73 to 82	16	6	66 to 94	0.53	May 17 to June 12
Check, no mulch	73 to 97					
12 inches lemon grass	74 to 87	13	3	67 to 97	.20	June 28 to August 20
Check, no mulch	74 to 100					
12 inches cane leaves	74 to 89	10	6	64 to 95	.32	August 20 to November 13
Check, no mulch	74 to 90					

[1]During the same hour.
[2]Depth of the temperature sensitive unit below the soil surface.

Air temperatures were often 7° to 10° F. lower than the lowest soil temperatures recorded. The highest soil temperatures beneath the mulches were always lower than the highest air temperatures. In contrast, soil temperatures of check plots without mulch were often higher than air temperatures. The soil temperature beneath mulched plots was sometimes 16° lower than the soil temperature of check plots at the same hour. Soil temperatures at a depth of 3 inches were in general a few degrees higher than at a depth of 6 inches. However, this difference may have been caused to some extent by corresponding higher air temperatures. Soil temperatures under cane leaves from August 20 to November 13 never were as high as earlier measurements although the air temperatures were in a comparable range. The even distribution of rainfall during this period may have been a contributing factor. Also, the second application of mulch during the first week of August no doubt was a factor in maintaining lower temperatures within these plots. Both kinds of mulch resulted in a narrower range of soil temperatures which never became as high as temperatures of the check plots. These differences may be within a critical range which will effect the development of derris roots and rotenone formation.

Mulched Derris required less weeding.—The mulching of *Derris* with cane leaves resulted in the greatest saving of labor required for weeding. Most of the weeding was necessarily done by hand to control nutgrass, *Cyperus rotundus* L. Less than half the man-hours spent in weeding check plots was required to maintain the plots mulched with cane leaves. Apparently 6 inches of cane leaves were as efficient in preventing weed development as 12 inches. Sometimes sprouts of cane arose, but these were easily pulled at an early stage. Lemon grass leaves did not control weed growth as well as cane leaves but, even so, such plots required considerably less labor than check plots. Twelve inches of lemon grass gave better weed control than 6. Derris debris (rotted leaves and stems) did not control weeds and apparently the mulch itself contained many weed seeds usually not present in the field. However, 6 inches of derris debris seemed to smother weeds of lower depths. In either case, derris debris cannot be considered a satisfactory mulch from the standpoint of weed control.

RELATIVE TOXICITY OF ROTENONE PLANTS

By Merriam A. Jones, Caleb Pagan, Edward R. McGovran,[2]
Wilbur A. Gersdorff,[2] and P. G Piquett[2]

Toxicity of derris and lonchocarpus not correlated with rotenone content.—In previous experiments a toxicological comparison of several types of rotenone-producing plants was described (*15*). The samples tested were of equal rotenone content but their toxicological values were found to be different. For example, the Sarawak Creeping variety of *Derris elliptica* was found to be about twice as toxic to houseflies as *Lonchocarpus utilis* A. C. Smith, although both were about the same in rotenone content. Another experiment involving comparison of these rotenone sources was carried out to check the previous results and to complete the data. In this work new samples were taken, the toxicological trials were made on three insect species, and chemical analyses of the roots were made for several constituents.

The varieties of *Derris elliptica* used were: Sarawak Creeping, St. Croix, and Changi No. 3. Sarawak Creeping was represented by two samples, one of plants grown in Puerto Rico and the other of plants grown in Guatemala. The Changi No. 3 was represented by samples of the Río Piedras and the Mayaguez-Goodyear clones. Two species of *Lonchocarpus* used were *L. utilis* and *L. chrysophyllus* Kleinh. Of the *L. utilis* one sample was from plants grown in Puerto Rico and one from plants grown in South America. This gave a total of 8 samples each consisting of 10 or more plants. The toxicological tests were made on houseflies,[3] Mexican bean beetles,[4] and cattle grubs. The results obtained with the latter insect are not included in this report.

As in previous work on the comparison of the toxicity of such roots, samples of extracted material corresponding to approximately 2 gm. of root powder were dissolved in acetone and diluted so that 1 ml. of the test solutions contained a little less than 0.1 gm. of rotenone.

In the tests on the Mexican bean beetle a technique different from that for the housefly was used.[4] Excised bean leaves sprayed with test solutions of the samples were placed in screened petri dishes with 10 third-instar Mexican bean beetle larvae. After 8 days the treated leaves were replaced by an unsprayed leaf to allow recovery of any larvae only weakened by the treatment. This technique approximated field conditions. The consumed areas of the sprayed leaves and the final mortality counts were averaged over 20 tests.

The data presented in table 2 show that the toxicities of the samples were about the same whether determined with acetone extracts on the housefly or with the diluted powder on the Mexican bean beetle.

Although considerable variations were encountered in the Mexican bean beetle test, there appears to be remarkably close agreement in the results of the toxicological tests with the two species of insects tested. The *Derris elliptica* Sarawak Creeping root grown in Puerto Rico was

[2] Entomologists of the Bureau of Entomology and Plant Quarantine.
[3] Gersdorff, W. A., and McGovran, E. R. Bureau of Entomology and Plant Quarantine Special Report No. 30. October 29, 1945.
[4] McGovran, E. R., and Piquett, P. G. Bureau of Entomology and Plant Quarantine Quarterly Spec. Rpt. No. 33. January 7, 1946.

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TABLE 2.—*The toxicological value and chemical analyses of some Derris and Lonchocarpus samples*

Material and location	Plants represented	Total chloroform extractives	Rotenone	Rotenone plus rotenoids	Alkali-soluble fraction	Fats and waxes	Neutral resin	Deguelin	Rotenone equivalent[1]		Mortality per square centimeter consumed[3]
									of the root	of the rotenoids[2]	
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
D. elliptica, Sarawak Creeping, Puerto Rico.	162	16.4	5.4	12.7	0.32	1.41	10.49	0.16	13.14	7.74	31
D. elliptica, Sarawak Creeping, Guatemala.	80	12.6	5.3	9.9	.33	.89	7.08	......	8.54	3.24	13
D. elliptica, Changi No. 3, Río Piedras clone, Puerto Rico.........	129	7.8	3.7	6.3	.34	1.43	3.86	......	6.27	2.57	18
D. elliptica, Changi No. 3, MG Clones, Puerto Rico...............	191	16.6	7.3	13.5	.48	2.45	7.31	.22	12.24	4.94	19
D. elliptica, St. Croix, Puerto Rico.........	854	5.9	1.8	4.6	.37	1.13	3.81	.13	4.73	2.93	21
L. utilis, Puerto Rico...	10	8.3	5.5	6.4	.22	1.97	2.43	....	8.54	3.04	6
L. utilis, South America.	32	12.4	4.7	8.8	.67	1.73	4.02	2.64	8.95	4.25	10
L. chrysophyllus, Puerto Rico...............	10	7.7	5.7	6.2	.37	1.05	2.06	....	6.84	1.14	5

[1]As determined by toxicity to houseflies.
[2]Total rotenone equivalent minus the rotenone content.
[3]As determined by toxicity to the Mexican bean beetle.

the outstanding insecticidal material according to its toxicity to both test insects. However, its rotenone content was by no means the highest. The same species grown in Guatemala was considerably less toxic. The root of *D. elliptica* Changi No. 3 Río Piedras clone, which was rather low in rotenone, was also low in toxicity to the housefly but appeared to be fairly toxic to the Mexican bean beetle. The sample highest in rotenone, *D. elliptica* Changi No. 3 MG clones, was second in toxicity to the housefly and third in toxicity to the bean beetle. The root of the St. Croix *D. elliptica* was low in rotenone and low in toxicity to the housefly but second in toxicity to the bean beetle.

The *Lonchocarpus* samples were generally less toxic than those of *Derris*. Here again the location of the planting appeared to influence the toxicological value of the root. The root of *L. utilis* grown in Puerto Rico was somewhat higher in rotenone content than that grown in South America, but the plants grown in Puerto Rico were less toxic to the housefly and even less toxic to the bean beetle. *L. chrysophyllus* root had very little toxicity other than that due to its rotenone content.

In these samples the toxicity resulting from rotenoids could not be accounted for by the content of neutral resins or of deguelin. In some of the samples, high total chloroform extractives were accompanied by high rotenone equivalent of the rotenoids, but this was not always the case.

The toxicological property of the roots was not always proportional to the amount of rotenone present and it is apparent that there are other constituents contributing to the insecticidal value of the material.

CHEMICAL INVESTIGATIONS

By Merriam A. Jones and Caleb Pagán

Sealing derris and lonchocarpus roots in jars reduced loss of rotenone.—These experiments were conducted with fresh roots of *Derris elliptica,* variety Sarawak Creeping harvested after having grown 2 years in the field. Roots 3 to 10 mm. in diameter were cut into pieces 20 mm. long. Duplicate samples, sealed and unsealed in mason jars, were stored for periods of 0, 2, 4, 8, and 12 weeks. Other treatments consisted of storing the roots for 12 weeks after the following treatments: (1) Sterilized in an autoclave 15 minutes at 120° C.; (2) treated with 1 ml. of toluene; and (3) stored unsterilized under a water-seal device. Analyses for moisture, total chloroform extractives, rotenone, and rotenone plus rotenoids were made at the end of each storage period. After 10 days the roots in unsealed mason jars were coated with a white mold, but none of the roots in sealed jars was moldy. Some of the samples developed adventitious roots during storage and these were subdivided into two samples, one having new roots and the rest having no new roots.

The presence of mold on the roots had no measurable effect on losses of dry matter, total extractives, or rotenone. The only samples that developed adventitious roots were those stored unsealed without further treatment. Unsealed material lost up to one-half of its original amount of rotenone within 12 weeks. Sterilization reduced rotenone loss about 50 percent in the unsealed samples, but did not prevent loss of dry matter. Dry matter losses in the unsterilized sealed samples may be ascribed to respiratory activity of the living cells. When the material was stored under these conditions for 12 weeks, the loss amounted to about 14 percent. Sterilization in the autoclave killed the tissues and stopped metabolic processes, but toluene treatment, as expected, had little effect on enzymatic action and consequently the loss of dry matter was about the same as in the unsterilized sealed material. Unsealed samples stored for 12 weeks lost approximately one-third of the dry matter and about half of the rotenone. This material was black and decomposed.

The trends of total extractives followed a similar pattern. The outstanding fact as regards rotenone values was that the losses were negligible in the samples that were sealed during storage. These samples did not darken appreciably and they developed a fruity acid odor unlike that of the resinous smell characteristic of fresh roots. Treatment with toluene did not affect sealed material, but sterilization in an autoclave apparently destroyed a small amount of rotenone. In the unsealed samples the rotenone losses increased with time of storage and were somewhat greater in samples with adventitious roots than in the corresponding samples without such roots. The material sterilized in an autoclave lost less rotenone during storage than did that not sterilized. These last two observations indicate that part of the rotenone loss during long storage may have been caused by oxidase activity, but the major portion of the loss was apparently due to decay which was not inhibited by the treatment with toluene.

A similar experiment was carried out with roots of *Lonchocarpus utilis* A. C. Smith, that had grown in the field for 7 years. Duplicate composite samples were stored for 0, 4, and 12 weeks, sealed and unsealed, with and without toluene.

The results obtained were in agreement with those for derris roots. The sealed samples lost little dry matter and even less rotenone while the unsealed material lost considerably more. Again, the pieces on which adventitious roots developed lost more rotenone than the corresponding pieces that did not develop adventitious roots. The toluene treatment was totally ineffective and the rotenone losses appeared to increase in 12 weeks of storage. The unsealed samples stored for 12 weeks were so badly decomposed that no adventitious root development could be observed.

It has been shown in previous reports (*17*) that sun-drying of derris and lonchocarpus roots is the most practical method of preserving them. It is rapid and results in no loss of rotenone. Shade-drying, even when fairly rapid, is less practical. Slow-drying, as simulated in these storage trials, is decidedly deleterious since considerable rotenone losses ensue unless the material is sealed.

Lonchocarpus root was sun-dried without loss of rotenone. —It has been generally accepted that roots of *Lonchocarpus* should be dried in shade to avoid loss of rotenone that would result from exposure to the sun. Growers and processors frequently state that lonchocarpus root must be washed and stored on shelves in a large structure with thatched roof and sides like that used for tobacco curing.

In previous studies it was shown that derris root, whole or split, could be dried in the sun, shade, or oven without degradation (*17*). This type of experiment was repeated with roots of *L. utilis*. Fresh roots, 3 to 10 mm. in diameter, were washed and cut to 2.5-cm. pieces. After being thoroughly mixed, duplicate composite 250-gm. samples were subjected to the following treatments: (1) Control, oven-dried immediately; (2) dried in the sun; (3) dried in the shade; (4) split to 2 mm. lathes and sun-dried; and (5) split and shade-dried. Duplicate moisture determinations showed the fresh root to have 63.1 percent moisture. The control was dried at 80° C. for ½ hour and then at 50° for two 4-hour periods until air-dry. Samples dried in the sun were exposed daily for 5 to 9 hours, while those dried in the shade were kept on a shelf out of direct light.

Split roots exposed to full sunlight were completely air-dried after 2 days, whereas whole pieces, dried in the sun required 4 days. Whole roots kept in the shade were dry in 2 weeks, but the split material dried at a somewhat faster rate.

The results showed that dry matter losses occurred only when the roots were dried in the shade. Those dried whole lost about 6.8 percent of their original dry weight while those split before drying lost 5.5 percent. However, these losses of dry matter increased the concentration of extractives and rotenone. When the results were reduced to an absolute basis there was actually no loss of extractives of rotenone either by sun- or shade-drying of whole or split pieces. This experiment indicates that lonchocarpus root, as well as derris root, can be dried in the sun without danger of loss in quality.

A microanalytical procedure for rotenone was devised.—The advantages of a microchemical procedure over a macromethod area: (1) Smaller samples may be analyzed, and (2) a saving of time, equipment, and materials is effected. The principal feature of a micromethod is that the scale of operations is reduced to one-tenth or less that of the

macroprocedure without excessive loss of precision and accuracy. A micromethod for rotenone analysis was set up using the principles of the A.O.A.C. method. This involves extraction with chloroform, precipitation of the rotenone as the carbon tetrachloride solvate, and reprecipitation from alcohol. By using the same principle for the micromethod, steps that might be questionable are avoided. The unique feature of the procedure is that, after extraction, all the manipulation is done in the same vessel, consisting of a microbeaker with a sintered glass filter. This device resembles a sprinkling can in that it has a receptacle to hold the liquid and on top has an opening and also a spout. The glass filter is sealed in the spout. The beaker has a capacity of about 7 ml. By using this apparatus, transfers of solutions and of precipitates are avoided.

The procedure was tested with pure rotenone and with derris resins at several concentrations. When duplicate rotenone samples were tried using 30, 60, and 90 mg. in the microbeakers, the solvate was about 74-percent rotenone and the recovery upon purification with alcohol was 29.9 and 30.3 for the 30-mg. samples, 60.1 and 60.2 for the 60-mg. samples, and 91.3 and 90.1 for the 90-mg. samples. The results were considered good as the crystals were comparatively large and clean.

When derris samples were used, the results were more erratic because the crystallization was not so complete, the presence of the other resins caused some error, and a small amount of rotenone passed through the sintered glass disk. With a derris sample of 6.8 percent of rotenone, five determinations gave 6.4, 6.6, 6.7, and 6.7.

CHEMICAL EXAMINATION OF MAMEY SEED

By Merriam A. Jones and Harold K. Plank

The insecticidal principle in mamey seed was shown to be an ester.—A previous report pointed out the similarity and contrast between the insecticidal principle in mamey seed and that in pyrethrum flowers (*18*). In recent work, further comparison between these two insecticides has been made. Barthel *et al* reported that the toxic principle of pyrethrum flowers could be extracted from the petroleum ether extractives by taking them up in a petroleum fraction and washing this with nitromethane (*3, p. 121*). After three extractions, the nitromethane solution was passed through a column of carbon and the solvent removed. It was found that 90 percent of the original pyrethrins was thus obtained and this material was 98-percent pure pyrethrins. In carrying out a similar procedure with mamey seed, it was found that not so much of the toxic principle was extracted with nitromethane and that about one-third of the resulting resin was hydrolyzable to a monobasic weak acid. This work indicated that the toxic principle is an ester.

An aliquot of the purified nitromethane extract of the petroleum ether solution was analyzed for methoxyl content. The result was so low that it was clear that the toxic principle contained no methoxyl groups.

PLANT TOXICOLOGICAL STUDIES

By Harold K. Plank

Several plant species showed varied toxicity to economic insects.—During the year, an additional order of insects, Orthoptera, making four in all, was included in laboratory tests to determine the insecticidal properties of various plants. At first the American cockroach (*Periplaneta*

americana (L.)) was used, but because of heavy parasitization of the egg-cases by *Tetrastichus hagenowii* Ratz., a change was made to the Australian cockroach (*P. australasiae* (F.)), which was parasite-free, took less time to rear, and being somewhat smaller was easier to handle. These were reared in quantity, and the usual test methods, previously outlined (*20, p. 15*), were modified to include these insects. Five last-stage nymphs were thoroughly dusted with 0.1 gram of the powdered plant material. After this treatment the roaches immediately began to clean their appendages by drawing them through their mouths. Each material thus had the opportunity to act as a stomach poison as well as a contact insecticide. The half-pint glass-jar cages with loosely fitting lids (five replicates for each material and the control) were stored in the dark. No food or water was given the roaches in these jars, and mortality readings were made at the end of 48 hours and 2 weeks.

The same technique was used with adults of the cottain stainer, *Dysdercus sanguinarius* Stal, but mortality was recorded only at the end of 48 hours. This change reduced the time of handling and possible mechanical injury and gave results as dependable as the former petri-dish technique.

When tested against either the American or Australian cockroach, or both, most of the plant parts previously reported (*22, pp. 22-23*) showed 4 percent or less toxicity at the end of 48 hours and 14 percent or less at the end of 2 weeks. Powdered seeds plus pods of *Pachyrhizus erosus* (L.) Urban var. A (P.I. 88365) had a toxicity of 13 and 32 percent at 48 hours and 2 weeks, respectively, while mamey-seed dust (*Mammea americana* L.) was 28 and 50 percent toxic at the same intervals. For comparison, a derris-talc mixture containing 0.5 percent of rotenone showed a toxicity of 17 percent in 48 hours and the same in 2 weeks, while a half-and-half mixture of technical sodium fluoride and wheat flour caused over half of the roaches to turn on their backs in 4 hours and all of them to die in less than 20 hours.

Tests made of additional plants as powders against one or more common representations of four orders of insects, including cockroaches, showed no particularly promising insecticidal properties. In these tests various plant parts were used, including fruit, seeds, leaves, petioles, spines, bark, wood, and roots. The following species were tested: *Albizzia stipulata* (Roxb.) Boiv., *Aleurites trisperma* Blanco, *Balanites aegyptiaca* (L.) Delile, *Caladium* sp., *Clibadium erosum* (Sw.) DC., *Clusia rosea* Jacq., *Commelina elegans* H.B.K., *Dieffenbachia seguine* (Jacq.) Schott., *Erythrina* sp. (P.I. 109849). *Piscidia piscipula* (L.) Sarg., *Solanum ciliatum* Lam., and *S. nigrum* L. The seed of *Albizzia stipulata* was the only part of this plant which showed any degree of toxicity to more than one species of insect, 52 percent to *Diaphania hyalinata* (melonworm) and 69 percent to *Dysdercus sanguinarius*. The roots of *P. piscipula* killed all the *Diaphania* larvae and 52 percent of the adults of *Dysdercus* used. In comparison, a derris-talc dust containing 0.5 percent of rotenone killed 80 percent of the *Diaphania* larvae, all of the adults of *Dysdercus*, and none of those of *Cerotoma ruficornis* (Oliv.).

The flowers and roots of mamey were only moderately toxic, killing 72 and 71 percent, respectively, of the *Diaphania* larvae and 8 and 56 percent, respectively, of the *Cerotoma* adults. Bark and wood from large limbs exhibited toxicities of 36 percent and less. A comparative test of the extractives from old, brown flowers and freshly opened flowers was

made in cooperation with M. A. Jones of this station. Extraction was accomplished by soaking in petroleum ether for 2 weeks and filtering. The old flowers yielded 0.45 percent extractives and the fresh, 0.26 percent, fresh-weight basis. At 1-percent restoration in inert marc the toxicities to *Diaphania* were 46 and 63 percent, respectively. Apparently the toxic principle was present in about equal amounts in both kinds of flowers, but the extractives were small in amount and of comparatively low toxic value.

Mamey-seed dust controlled fleas and ticks.—One of the earliest uses of mamey in the West Indies has been in the control of insects, particularly chigoes ("niguas") and other fleas, attacking man and domestic animals (*13, pp. 82-83*).

The effectiveness of the powdered seeds for such use was recently verified by dusting 2 dogs and 70 laboratory white mice 10 months old, moderately infested with fleas of undetermined species. A liberal dusting of the dogs by rubbing the powder into the hair, particularly over the neck and back, resulted in a high kill of fleas and caused all ticks present to drop within 24 hours. The fleas were controlled on the mice also, but 4 of the dusted mice died within a week. Death was apparently due to other complications than skin abrasions, as none of 10 artificially abraded mice died.

Infusion of immature mamey fruits was highly toxic to certain insects.—It has been shown that the immature fruits of mamey, when dried and powdered, possessed little effect as an insecticide (*20, p. 15*). Immature to half-ripe fruits were tested in the manner recorded by Grosourdy (*13, pp. 82-83*) and said to be sometimes employed locally by small farmers, namely, as an infusion in water. Applied as a stomach poison, this infusion killed all the *Diaphania* larvae used and 4 percent of the *Cerotoma* adults; as a contact poison it was 90 percent toxic to *Diaphania*, 100 percent to *Cerotoma* adults, and 57 percent to *Dysdercus* adults. Toxicity is apparently due to the gummy sap, which readily diffused in the water, for, when this settled, the supernatant liquid was practically inert both as a stomach poison and by contact on *Diaphania* larvae and *Cerotoma* adults.

As a contact poison, the above infusion produced complete mortality of the fleas, *Ctenocephalides felis* (Bouché) and *Pulex irritans* L.,[5] in ½ hour. This preparation, which can be readily made from locally available material, showed promise as a practical wash for the control of fleas on dogs and perhaps other domestic animals.

DRUG-CROP INVESTIGATIONS

CINCHONA PROPAGATION

By Harold F. Winters

Cinchona field plantings expanded.—The 1945 field plantings of *Cinchona* were considerably delayed due to lack of sufficient rainfall in July and August. During the last half of July only 0.58 inch of rain was recorded in the Toro Negro National Forest with a total of 7.17 inches for the month. Rainfall for August was 12.49 inches, but since 9.40 inches of this fell on the third and fourth days of the month, the last

[5] Determined by C. F. W. Muesebeck, Bureau of Entomology and Plant Quarantine.

5 weeks were almost without rain. During September the situation improved considerably, with some rain falling almost every day.

A total of 3,790 trees were planted to the field during the year. Two lots of trees consisted of progeny from earlier plantings in Puerto Rico and others represented importations of seed or plants. Table 3 gives a detailed list of the various strains planted, with the source and number of each.

TABLE 3.—*Cinchona field plantings made during 1945 in the Toro Negro National Forest*

Species or strain	Source of seed	Date planted	Number planted
Cinchona crosses	Planting in Maricao Forest	Nov. 8, 1945	55
C. ledgeriana Moens[1]	P.I. No. 143981[2]	Sept. 19-Oct. 31, 1945	1,188
C. ledgeriana	Guatemala, principally Coban strain	Oct. 19-25, 1945	173
C. ledgeriana	Planting in Maricao Forest	Oct. 26, 1945	262
C. ledgeriana	P.I. No. 148115[2]	Nov. 1945	630
C. pubescens Vahl	Castañer PRRA Farm Adjuntas, Puerto Rico	Sept. 27-Oct. 10, 1945	226
C. ledgeriana	P.I. No. 144071[3]	Nov. 7, 1945	48
C. officinalis L. No. 45	P.I. No. 144073	Oct. 31, 1945	28
C. pubescens No. 22	P.I. No. 144079	Nov. 9, 1945	8
C. pubescens No. 22	P.I. No. 144080	Nov. 9, 1945	182
Cinchona sp. X—2, S. Brazil	P.I. No. 144082	Nov. 8, 1945	47
Cinchona sp. X—3, S. Brazil	P.I. No. 144083	Nov. 8, 1945	94
Cinchona Hybrid No. 9	P.I. No. 144084	Nov. 1, 1945	87
Cinchona Hybrid No. 11	P.I. No. 144085	Nov. 1, 1945	93
Cinchona Hybrid No. 147, S. Brazil	P.I. No. 144086	Nov. 8, 1945	25
C. officinalis ledgeriana	P.I. No. 144093	Oct. 31, 1945	20
C. officinalis ledgeriana	P.I. No. 144095	Oct. 31-Nov. 7, 1945	100
C. officinalis ledgeriana	P.I. No. 144096	Oct. 31, 1945	56
C. officinalis ledgeriana	P.I. No. 144097	Nov. 7, 1945	25
C. officinalis ledgeriana	P.I. No. 144098	Oct. 31, 1945	11
C. officinalis ledgeriana	P.I. No. 144099	Oct. 31, 1945	15
Cinchona Hybrid Kaatoan Coll.	P.I. No. 144106	Nov. 2, 1945	109
Cinchona Hybrid Impalatao	P.I. No. 144107	Nov. 2, 1945	32
Cinchona Hybrid No. 1	P.I. No. 144108	Nov. 9, 1945	15
Cinchona Hybrid No. 3	P.I. No. 144110	Oct. 31, 1945	45
Cinchona Hybrid No. 10	P.I. No. 144111	Nov. 9, 1945	4
Cinchona Hybrid No. 11	P.I. No. 144112	Nov. 2, 1945	14
Cinchona Hybrid No. 168	P.I. No. 144113	Nov. 9, 1945	57
Cinchona Hybrid X—4	P.I. No. 144114	Nov. 9, 1945	14
Unidentified Fischer selection		Nov. 7-8, 1945	127

[1] The systematic position of *Cinchona ledgeriana Moens* is uncertain; most likely it is conspecific (perhaps a variety) of *C. officinalis* L., but this matter has not been settled.

[2] Material received as nursery seedlings from the Bureau of Plant Industry, Soils, and Agricultural Engineering, Glenn Dale, Md., in cooperation with the Defense Supplies Corporation.

[3] Material received as seed from the same source as seedlings.

Growth and survival of Cinchona seedlings not affected by removal of sphagnum from roots.—In a preliminary experiment in 1943 with imported *Cinchona ledgeriana* seedlings grown in sphagnum moss a study was made of different methods of handling the plants in outdoor nurseries. The results of this experiment showed no significant differences between treatments. Since this trial was of an observational nature and the results inconclusive, the experiment was repeated on a more extensive scale with several replications of each treatment as follows: (1) *C. ledgeriana* seedlings with the sphagnum ball left intact, (2) seedlings from the

same source with sphagnum ball removed by washing, and (3) *C. ledgeriana* seedlings grown locally in soil and transplanted with soil removed from roots.

In view of the fact that soil moisture and aeration might prove an important factor in the survival and growth of these seedlings, the number of treatments were increased by crossing the above treatments with degree-of-watering tests as follows: (1) Light watering—water applied only when necessary to avoid wilting; (2) medium watering—1 gallon per plot per week; and (3) heavy watering—2 gallons per plot per week or until soil appeared saturated. While this may appear to be a small amount of water to attain the desired degrees of soil moisture, the heavy shade of the palm shelters, the canopy of *Inga vera* Willd., and the protection from wind reduced evaporation considerably.

The desired water levels were satisfactorily maintained during the dry season or from late November to late February. With the advent of the spring rains in March, however, all the plots became saturated at occasional intervals.

The experiment was concluded in September 1945 when data were taken on survival, height, and number of diseased plants.

An analysis of variance showed that survival of the *Cinchona* seedlings grown locally in soil was better than that of imported sphagnum-grown seedlings. The difference in survival between treatments of imported seedlings was not significant. Locally grown seedlings had significantly fewer diseased plants at the end of the experiment than the imported seedlings. Differences between treatments of imported seedlings from the standpoint of disease were not significant, although the greatest number of diseased plants were found where the moss was removed from the roots. There were no important differences in height.

In the degree-of-watering treatments, survival was best in plots of medium soil moisture, next best in plots with high soil moisture, and lowest in the lightly watered plots. None of the differences, however, attained significance. Incidence of disease was significantly lower in the medium-water treatment than in the others. Disease in the heavily watered plots was somewhat lower than in the lightly watered plots.

Selection of large- and medium-sized seedlings gave best results.—A group of imported *Cinchona* seedlings was sorted into the following sizes and planted separately in plots replicated four times: (1) Large seedlings, from 6 to 8 inches, (2) medium seedlings, from 4 to 6 inches, and (3) small seedlings, from 2 to 4 inches. After 9 months in the nursery, data were taken on height, survival, and incidence of disease.

Survival was best in the medium-sized plants, second best among the large plants, and poorest in the small plants, the difference in survival between medium and large plants not being significant. Growth of both medium and large seedlings was better than growth in the small class, the differences being of a high order of significance.

The incidence of disease among small plants, 27.7 percent, was significantly higher than among large- or medium-sized groups in which there were 15.4 and 16.0 percent diseased, respectively.

At the end of the experiment the large plants had an average height of 9.0 inches, the medium-sized plants 8.0 inches, and the small plants averaged 5.75 inches. All differences were highly significant.

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It would seem advisable on the basis of these results to discard small plants from a mixed lot of seedlings. The plants grown from small seedlings continue to remain small, are more susceptible to disease, and have less chance of survival than larger plants.

Arrangement of seedlings in nursery beds was important.—Experience has shown that there are distinct differences in size and vigor between plants grown in the front and those grown in the back of standard nursery beds. This is apparently due to reduced light intensity at the rear of the beds. An experiment was conducted to determine the effect of position of the plants in the beds on the ultimate size of large, medium, and small transplants. Treatments were as follows: (1) Small plants at front of bed, 2 rows; medium plants in middle of bed, 2 rows; and large plants at the rear, 1 row; (2) the reverse of the above treatment with large plants at front of bed; and (3) large, medium, and small plants planted at random. The rows were numbered 1 to 5, front to back, running lengthwise of the bed.

Recovery of all types of seedlings after transplanting was good and at first there was little difference in vigor between plants at the front or back of the beds. Within a few months, however, the plants in the front rows were growing more vigorously than those in the back rows. During the final months of the experiment plants in the back rows made very little growth. Where the small plants were planted at the front, medium-sized plants in the middle, and large plants at the back of the nursery bed, survival was 84 percent as compared to 78.7 percent in each of the other treatments. Although the differences between treatments in average survival and plant height were not statistically significant, there was in reality considerable difference in size and appearance of the plants within treatments. The interaction between whole-plot and within-plot data was highly significant. Treatment 1 with small plants at the front produced the most uniform plants both in height and survival.

In treatment 2, where the small plants were planted at the back and large plants at the front, the large plants retained their advantage throughout the experiment. The height of plants in rows 3, 4, and 5 increased very little during the experiment over the average height at planting time. The exclusion of light caused by greater height of plants at the front of the bed was apparently another factor contributing to this marked differential in growth. Survival of seedlings in rows 1, 2, and 3 was significantly better than in rows 4 and 5.

Where plants of various sizes were planted at random, the seedlings in row 1 again made the best growth. Plants in rows 1, 2, and 3 survived better than those in rows 4 and 5.

More uniformity in survival and height may be secured by sorting the plants and placing the large ones at the back, medium-sized ones in the middle, and small plants at the front of the nursery bed. This, of course, would only hold true where shaded nursery beds are used. This experiment further emphasized the need for a better-designed nursery bed which admits proper and uniform light throughout the bed.

Cuttings taken from young Cinchona seedlings rooted best.—Numerous trials have been made in an effort to root cuttings of *C. ledgeriana* and *C. pubescens*. The objective of past experiments has been primarily to develop a method of rooting cuttings from mature trees. Such a method would be of particular value in propagating individual

trees of high alkaloid content. Propagation of *Cinchona* by marcottage has been found possible during the rainy season, but it is a cumbersome process (*32*).

For these trials sand was used as a rooting medium. Various growth-promoting substances were employed in an effort to induce rooting. The alcoholic dip method of treating cuttings described by Cooper (*7*) was used in which the hormones were dissolved in 50 percent ethyl alcohol. The concentrations used were as follows: Indolebutyric acid, 2, 5, and 10 mg. per milliliter, and naphthalene acetic acid, 2 and 5 mg. per milliliter. The basal end of the cutting was held for 5 seconds in the alcoholic solution. A commercial dust treatment, Hormodin No. 3, was also used.

Shoot cuttings from mature trees were tested at three stages of development, soft, mature, and hard. In almost all cases where cuttings from mature trees were used with or without growth-promoting materials, the trials resulted in failure. The exceptions were a few treated juvenile or sucker growths taken from near the base of the mature trees. Five such cuttings, out of 37 tried, were successfully rooted. In many cases cuttings survived in the propagation case for 5 to 9 months before dying. Usually one or two pairs of new leaves developed during this time, but in the treatments using 5 and 10 mg. of indolebutyric acid there seemed to be inhibition of terminal growth. Many cuttings callused but did not produce roots.

Better results were obtained with cuttings taken from young seedlings. With cuttings taken from plants 8 months old, 77 rooted out of 114 planted, or 67.5 percent; nearly all of those which rooted grew into excellent plants for field planting. Where the stocks were 1 year of age, an average of 63.4 percent of the cuttings rooted. Fourteen percent rooted from 2-year-old seedlings. However, these latter cuttings were not exactly comparable as in some cases different types of cuttings were used. The average rooting for all trials including cuttings from mature trees was only 22.4 percent. There appeared to be a correlation between age of plants from which the cuttings were taken and their ability to root.

The use of growth-promoting substances in weak solutions gave a higher percentage of rooting in some experiments. However, the untreated cuttings usually rooted about as well, but not as rapidly, as treated cuttings.

In experiments with leafy and leafless cuttings, 33 percent of those with leaves produced roots, while only 9.3 percent of the leafless cuttings rooted. There is an advantage in leaving some foliage on the cuttings to induce rooting.

Soil fumigation with chloropicrin gave higher survival in Cinchona nursery beds.—*Cinchona* seedlings in Puerto Rico are often attacked by a disease similar to that caused by *Phytophthora* spp., as described by Crandall and Davis (*8*). The effectiveness of chloropicrin as a soil fumigant for the control of several genera of soil fungi has been demonstrated previously by Godfrey (*12*).

For this experiment an old nursery bed at Maricao was selected which contained a uniform soil high in humus. The previous planting of *Cinchona* seedlings was removed from the bed because of high incidence of disease. It was assumed that sufficient inoculum was present either in the soil or plant refuse to reinoculate the new planting.

The bed was divided into six plots by wooden partitions that allowed no direct contact between the soil of adjoining plots. Concentrated liquid chloropicrin was applied to alternate plots at the rate of 500 pounds per acre. Application was made directly into the soil by means of an injector manufactured for this purpose. After treatment the soil was watered and the treated plots covered with a tarpaulin for 2 weeks to aid in retaining the gas. One month later, 100 plants of *Cinchona ledgeriana* were planted in each plot. Those which failed to survive in the initial planting were replaced, after which losses were considered to be due to experimental conditions.

Stand counts were made after 3, 6, and 9 months and counts of diseased plants after 3 and 9 months. The results of these counts are shown in table 4.

TABLE 4.—*Effect of soil treatment with chloropicrin on height, survival, and disease of Cinchona ledgeriana plants*

Soil treatments	Average survival per plot			Average diseased per plot		Average height
	3 mo.	6 mo.	9 mo.	3 mo.	9 mo.	9 mo.
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Inches*
Chloropicrin: 500 lb. per acre.......	90.0	54.3	44.3	4.8	9.4	9.52
Check: No treatment	61.0	23.0	16.7	7.7	4.4	4.34

An analysis of variance based on the data collected 9 months after planting showed the chloropicrin treatment, with a mean survival of 44.3 plants per plot, to be significantly better than the check with a mean survival of 16.7 plants per plot. Also, average height of the treated plants, 9.52 inches, was better by high statistical odds than that of the check plants, 4.34 inches. Plants in the treated plots generally had a more thrifty appearance than those in the check plots.

In this experiment the plots treated with chloropicrin proved to be definitely better than those untreated. While the average survival of 44.3 plants at 9 months in the treated plots was not good, there was a decided benefit from the use of chloropicrin. Other factors such as soil, wind, and even possible contamination of the treated plots may have been responsible for the low survival. In the event it is necessary or desirable to replant nursery beds in which the previous *Cinchona* plants were diseased, soil treatment with chloropicrin is suggested for improving the stand.

PHYSIOLOGICAL STUDIES

By HAROLD F. WINTERS, ARNAUD J. LOUSTALOT, AND NORMAN F. CHILDERS

Growth responses of Cinchona differed under three temperature ranges.—In March 1945 an experiment was initiated to study the effect on growth and alkaloid content of two species of *Cinchona* grown under three different temperature ranges. Temperature differentials, as shown in table 5, were obtained by the use of three thermostatically controlled air-conditioned chambers built within a standard greenhouse.

TABLE 5.—*Average height, weight, and survival of Cinchona ledgeriana and C. pubescens seedlings grown at three temperature levels*

	Temperature[1]				Average dry weight per plant		Average height		Survival	
	Summer		Winter							
	Night	Day	Night	Day	*C. ledgeriana*	*C. pubescens*	*C. ledgeriana*	*C. pubescens*	*C. ledgeriana*	*C. pubescens*
	°F.	*°F.*	*°F.*	*°F.*	*Gram*	*Gram*	*Centimeter*	*Centimeter*	*Percent*	*Percent*
Warm chamber........	70	80	65	80	5.48	18.73	54.2	74.6	86.4	56.8
Medium chamber......	65	75	60	75	8.36	13.26	70.6	67.5	90.0	82.5
Cold chamber..........	60	70	55	70	7.13	9.05	46.7	45.2	85.0	100.0
Greenhouse bench......	No control				4.23	.....	36.8	....	57.5	....

[1]Summer temperatures maintained from April through September and winter temperatures from October through March.

The experiment was concluded in February 1946, when final observations and growth data were taken. The two species of *Cinchona* responded quite differently to the temperature treatments. In the warm chamber *C. ledgeriana* produced weak slender stems and poor root systems. During the summer many necrotic spots developed on the leaves. A more vigorous healthy growth was produced in the medium-temperature chamber, while in the cold chamber, a stocky growth developed characterized by thick stems, vigorous roots, and undulate red-tinged leaves. Outside the chambers in the greenhouse, where temperatures averaged 5° to 10° F. higher than the warm chamber, growth of *C. ledgeriana* was unsatisfactory. Many large necrotic areas developed on the leaves during the summer and subsequent leaf shedding left the stems almost bare. Root development was extremely poor.

The data presented in table 5 show that the *Cinchona ledgeriana* seedlings grown in the medium-temperature chamber made the most vigorous growth as measured by height and dry weight. The seedlings grown in the warm chamber were taller on the average than those in the cold chamber but had an average dry weight less than that of the cold-chamber plants. Plants in the outer greenhouse were inferior to all plants grown in the chambers. All differences were statistically significant at the 0.05 level and in some cases at the 0.01 level. There was no appreciable effect of treatment on the concentration of dry matter in plants of *C. ledgeriana.*

Temperature had a definite effect on the shoot-root ratio, the seedlings in the warm chamber having the highest ratio and those in the cold chamber having the lowest.

The percentage of *Cinchona ledgeriana* plants surviving from the original number planted was approximately the same in all chambers. The relative proportion of leaves to the rest of the plant was not appreciably different in any of the treatments. However, the plants grown in the cold chamber had a lower percentage of stem than the plants grown in either the medium or warm chambers. On the other hand, stems of plants in the cold chamber had a higher percentage of bark than those grown in the warmer chambers.

There was no appreciable difference in the percentage of total alkaloids in the stems of plants in any treatment, but the percentage of quinine

in stems of seedlings grown under the medium-temperature conditions was somewhat lower than that of stems from the other treatments.

In the roots the percentage of total alkaloids was highest in plants grown in the warm environment and lowest in plants from the medium-temperature chamber, while quinine was somewhat higher in the roots of plants grown in the medium-temperature chambers.

Cinchona pubescens is morphologically quite different from *C. ledgeriana*. It is not surprising therefore that their response to temperature was considerably different. The most vigorous and best growth as shown by height and weight measurements was produced in the warm chamber. Growth of this species was directly correlated with temperature. The cold environment produced short stocky plants with short internodes and excellent root systems. Plants from the medium-temperature chamber were intermediate in size and vigor as compared to those from the warm and cold chambers. Percentage of survival was inversely correlated with temperature. In the warm chamber competition due to the large leaves and close spacing of the plants resulted in death of the weaker plants, while in the cold chamber where growth was slower 100 percent survived. This is in contrast to the uniform survival in all chambers of the narrow-leaved *C. ledgeriana* where competition for light apparently was not a factor. All differences were of a high order of significance. Unlike *C. ledgeriana,* temperature was significantly correlated with concentration of dry matter in *C. pubescens.* The plants in the warm chamber produced the highest percentage of dry matter and those in the cold chamber the lowest.

There was no significant effect of temperature on shoot-root ratio but the proportion of leaves to total plants on a dry-weight basis was lowest in the warm chamber and highest in the cold chamber while the reverse was true with percentage of stems to total plant.

As with *Cinchona ledgeriana,* the percentage of bark on the stems was somewhat higher on seedlings grown in the cold chamber than on those grown in the warmer chambers.

The percentage of total alkaloids in roots and stems and the percentage of quinine in the roots was directly correlated with temperature. The percentage of quinine in the stems of plants grown in the medium-temperature chamber was somewhat lower than that of plants grown in the warm and cold chambers.

The results obtained in this experiment emphasize the importance of temperature as a factor influencing the growth of *Cinchona*. A comparison of the responses of the two species to relatively small differences in temperature (5° to 10° F.) indicate that *C. pubescens* is better adapted to warmer temperatures whereas *C. ledgeriana* is more exacting in its requirements. Of the two species, *C. pubescens* was by far the most vigorous grower regardless of temperature conditions. This is probably because of its inherent ability to grow and produce vigorous root systems under varied conditions. Development of the root system of *C. ledgeriana* was adversely affected by the higher temperatures.

Although plants of *Cinchona ledgeriana* grown in the warm chamber made more linear growth than those from the cold chamber, the plants in the cold chamber had a greater average dry weight.

The beneficial effect of the cold temperature in producing a higher percentage of bark for both species was offset by the smaller total dry weight produced under these conditions.

CHEMISTRY

By Arnaud J. Loustalot and Caleb Pagán

A rapid semiquantitative method developed for estimating quinine.—In selecting a method for evaluating *Cinchona* bark, due consideration should be given to the kind of information required and its application. For example, a cinchona grower or plant propagator desiring to know the quinine content of his bark will require a less accurate and elaborate process than a manufacturer buying bark for the extraction of quinine. Most analytical methods in use at present require elaborate expensive apparatus and considerable time and skill in extracting and determining the quinine content of the bark. Usually the methods also require relatively large samples of 10 to 20 gm. Thus, there is a definite need for a simple, quick method of estimating quinine in small samples of *Cinchona* bark that can be carried out with a minimum of apparatus and equipment.

A review of the literature suggested that the thalleoquin reaction might be adapted for use in the semiquantitative estimation of quinine. Numerous tests were made using standard solutions of quinine sulfate and pulverized mahogany bark containing known added amounts of quinine sulfate. Various quantities of bromine and ammonia were then added to the solutions at different dilutions and under various experimental conditions. A combination of variables was found that indicated by color intensity the approximate concentration of quinine sulfate.

The method has the advantage of using a minimum of equipment and reagent and in requiring only small samples of bark (2 gm). It has the disadvantages of giving only an approximation of the quinine content, i.e., within 1 percent of the amount actually present, and it includes as quinine any quinidine or related substances that give the thalleoquin reaction. The method is most suitable as a quick "screenings" test for use by plant breeders or by cinchona growers desiring to cull their low quinine yielding strains without an elaborate and expensive chemical analysis of each sample.

Quick simple method developed for assaying Cinchona bark.—Although the thalleoquin method described above may be useful in certain instances, it was not satisfactory for determining small quantities of quinine as found in young seedlings. *Cinchona* bark containing less than 2 percent quinine gave little or no color under conditions of the test. Therefore, other methods of analyzing small samples of low quinine content were investigated.

In 1942 and 1943, Carol (*4, 5*) described a method for the quantitative determination of quinine by absorption spectrophotometry. The method is based on the fact that quinine salts have a strong absorption band in the near ultraviolet with a maximum absorption at approximately 340 mμ. The results obtained from analysis of commercial samples of quinine sulfate by absorption spectrophotometry at 34 mμ were good but as Carol points out, "Quinine can not be determined by this method in the presence of the other *Cinchona* alkaloids, as they have absorption bands at 340 mμ."

It was felt that if the photometer method could be modified and adapted for use with *Cinchona* bark samples and quinine determined in the presence of the other *Cinchona* alkaloids a great saving of time would be achieved. The separation of quinine from the other *Cinchona* bases

would be obviated and it would be possible to determine quinine directly in the extraction medium.

Experiments were carried out using dilute solutions of the *Cinchona* alkaloids, mixed and alone. The percentage transmittance of light at various wavelengths was measured in a Coleman double monochromator spectrophotometer, Model 105, using the 30 mμ slit in conjunction with a Coleman electrometer, Model 310, at a wavelength of 380 mμ. At this wavelength cinchonine and cinchonidine had no effect on the absorption of light. The absorption of light was proportional only to the amount of quinine and quinidine present.

Solutions containing quinine alone and quinidine alone at the same concentration gave equal transmission values at 380 mμ. Solutions of cinchonine and cinchonidine at different concentrations either alone or combined gave no readings at that wavelength. When the method was applied to samples of *Cinchona* bark containing the four *Cinchona* alkaloids in various proportions, the presence of cinchonine and cinchonidine had no measurable effect on the percentage of quinine and quinidine.

Since quinidine resembles quinine in the physiological effects, no serious error is incurred by its inclusion as an antimalarial constituent. Also, the proportion of quinidine to quinine is usually very small in most *Cinchona* barks.

An important step in devising a quick method of determining quinine in *Cinchona* bark was the necessity of developing a rapid, simple procedure for extracting the alkaloids from the bark quantitatively The *Cinchona* alkaloids are soluble in varying degrees in such organic solvent or in ethyl alcohol, ethyl ether, chloroform, and benzene.

Of the various solvents considered, ethyl alcohol appeared to be the most suitable since it is less volatile and less expensive than most others and the four major *Cinchona* alkaloids dissolve readily in it. Treatment of *Cinchona* bark with lime or other alkaline substances is known to separate the alkaloids from cinchotannic and quinic acid with which they are combined in the bark.

Tests were made of various techniques for extracting the alkaloids from the bark and the one that gave consistent and reproducible results and was simplest to carry out was as follows:

Two grams of dried (100 mesh or finer) *Cinchona* bark are treated with half a gram of finely powdered calcium oxide (the amount is not critical) and enough water (7 to 10 ml.) is added to make a smooth homogenous paste. After standing for 10 minutes the paste is transferred to a 200-ml. volumetric flask with 100 to 150 ml. of ethyl alcohol. The alcoholic bark suspension is well shaken and allowed to stand an hour or longer with occasional shaking (3 times) after which it is made to volume with alcohol, shaken and filtered through 24-cm. diameter Whatman No. 5 filter paper. A watch glass is placed over the funnel and a plug of cotton in mouth of Erlemeyer flask receiving the filtrate to minimize loss from evaporation of alcohol during filtration.

Quinine is determined directly in the extraction medium by the following procedure:

Twenty-five milliliters of the alcoholic extract are treated with 25 mg. of Norite A to remove coloring matter. To 8 ml. of the cleared extract, 5 ml. of tenth normal HCl is added and diluted to 100 ml. The percent transmittance at 380 mμ is measured in a spectrophotometer, and percent quinine is read directly from a curve prepared from transmittance values obtained from a series of standard quinine sulfate solutions.

Good agreement was obtained between samples of *Cinchona* bark analyzed by the official method and the spectrophotometer method described

above. In general, the quinine values agreed within 0.1 percent with an occasional deviation of 0.2 to 0.3 of a percent.

The spectrophotometer method has the advantage of requiring small samples of bark and can be used to determine small amounts of quinine. It is particularly useful in evaluating *Cinchona* seedlings for antimalarial constituents at an early stage in their life cycle. A large number of seedlings can thus be "screened" in a relatively short time, which is an important factor in facilitating such projects as breeding and selection programs. The method can also be used in assaying samples of *Cinchona* bark for commercial purposes.

Total alkaloids determined by titration.—Where strict accuracy is required, gravimetric methods are usually preferable to volumetric methods. This is particularly true in the case of the *Cinchona* alkaloids where the molecular weights are high. A small error in titration results in a large error in the amount of alkaloid found. However, in instances where large numbers of samples are involved and highly accurate results are not required, a great saving of time may be effected by the titration method. Accordingly, tests were conducted with aliquots of the alcoholic bark extract which included evaporating off the alcohol and dissolving the residue in various amounts of standard acids, filtering and titrating the excess acid with standard NaOH, using different indicators and a pH meter. A simple and quick method for estimating the *Cinchona* alkaloids was found by titrating 100 cc. of the acidified alcoholic extract (representing 1 gm. of *Cinchona* bark) with dilute NaOH and a pH meter. The values for total alkaloid obtained by titration were generally somewhat higher than those obtained gravimetrically. However, a good approximation of the total alkaloids in *Cinchona* bark can be obtained by the titration method and it has the advantage of being rapid and requiring relatively small samples.

FOOD-CROP INVESTIGATIONS

VEGETABLE INVESTIGATIONS

By Norman F. Childers, Harold F. Winters, Pedro Seguinot Robles, and Harold K. Plank

Heavy rainfall and high temperatures detrimental to vegetable production.—In January 1945, vegetable trials were initiated at Mayaguez (50 feet elevation), Maricao (2,000 feet), and Toro Negro (3,300 feet) to determine the adaptability of some 45 vegetables over a period of 2 years. Only those varieties were tested which had given satsifactory results with local growers or which had received favorable recommendation in the Caribbean area. Trials were completed for 24 vegetables ending about February 1946, at which time trials for the remaining vegetables were initiated for the following year. All plantings of a given set of vegetables were repeated at 2-month intervals over a 12-month period.

The mean monthly temperature and the amount of rainfall appear to be the chief factors governing the kinds and varieties of vegetables and the season when they can be grown best in Puerto Rico. Vegetables such as cabbage, head lettuce, kohlrabi, and broccoli which are a complete failure in July and August, especially at Mayaguez, can be grown successfully during the winter months, particularly from November to March, inclusive. The difference in mean monthly temperatures between

summer and winter is not great in Puerto Rico. For example, at Mayaguez during the season of 1945 the mean monthly temperature in January, the coolest month, was 73.5° F. as compared with 79.1° in July, the warmest month. This small temperature difference of 5.6°, however, apparently makes the difference between growing or not growing certain vegetables, as Frazier (*10, pp. 104-106*) has pointed out in Hawaii.

The amount of rainfall is also a factor definitely influencing the growth of all kinds of vegetables. From April to October 1945, 6.5 to 15.5 inches of rain fell per month with particularly heavy rains in May, August, and September. The excessively heavy rainfall during May, when over 13 inches of rain fell in 1 week, and about 3.5 inches on 2 successive days, resulted in poor growth and death of most vegetables at both Mayaguez and Maricao. Such a heavy rainfall concentrated in 1 week is disastrous to any vegetable planting. Rainfall during the winter months at all three altitudes is so low under normal conditions that some irrigation is necessary to produce satisfactory yields.

Poor yields or complete failures were obtained during the summer months at all three locations with cool season crops such as cabbage, Chinese cabbage, kohlrabi, and broccoli. Even the more heat-tolerant vegetables, such as tomatoes, leaf lettuce, and peppers, produced poorly in summer, largely due to the heavy rains which not only mechanically injure the foliage but keep the soil saturated for periods of as much as 4 to 7 days at a time.

Vegetables grew well at Mayaguez during winter season.—Vegetables which grew particularly well at Mayaguez during the winter months were Succession cabbage, Chi Hi Li Chinese cabbage, Rosita eggplant, Slobolt leaf lettuce, White Velvet okra, Giant Stringless Greenpod and Puerto Rican White 1320[6] bush beans, Melting Sugar pea, and Burpee's Earliest Scarlet Button radish. The Succession cabbage produced many large heads, some 6 to 7 pounds in weight, which is unusually good for Mayaguez conditions. Decidedly better cabbage was produced by transplanting the seedlings from the seedbed to 5-inch pots, and thence to the field. The new United States Department of Agriculture heat-tolerant Slobolt leaf lettuce produced some bunches 15 to 18 inches in diameter which were in greater demand on the station market than the Black Seeded Simpson variety. No bolting was evident at any time with Slobolt, whereas the Black Seeded Simpson variety showed varying degrees of bolting at the beginning and end of the winter season and heavy bolting during the summer. The best crop of bush beans grown in these trials was produced during January and February 1946. The continental variety, Giant Stringless Greenpod, led the 11 varieties tested; second best yielder was Puerto Rican White No. 1320. From the standpoint of labor involved and yield per unit of ground, Chi Hi Li Chinese cabbage was one of the most profitable vegetables grown; no trouble was encountered in selling the relatively large crop.

Vegetables which gave fair yields during the winter season were tomatoes, bush lima beans, Brown Sugar Crowder cowpea, Puerto Rico No. 39 cucumber,[6] and Black Zucchini squash. Cauliflower and celery were complete failures under the conditions of these trials.

[6] Developed by the Agricultural Experiment Station of the University of Puerto Rico, Río Piedras.

During the summer period at Mayaguez no vegetables were outstanding; most were failures. Vegetables which gave fair yields were White Velvet okra, New Long White Bunching onion, Scarlet Button radish Slobolt leaf lettuce, and Don Juan sweetpotatoes. Vegetables which gave poor yields were tomato, Mallorquin pepper, Kentucky Wonder pole bean, Melting Sugar pea, and Yellow Self-Blanching celery. Vegetables which were complete failures at Mayaguez included cabbage, head lettuce, lima beans, most varieties of bush beans, carrots, bulb onions, beets, turnips, cauliflower, Chinese cabbage, eggplant, and most varieties of peas, squash, peppers, and cucumbers.

Vegetables grew well at higher altitudes.—The vegetables at Maricao were planted on a Nipe clay which is one of the most unproductive soils in Puerto Rico (*24, p. 200*). Rainfall at Maricao is usually adequate at the beginning and end of the winter dry season, but excessive in summer. Some irrigation was provided during the winter periods, and manure and fertilizers were added to each planting in standard amounts. Apparently because of the slightly lower mean temperature at Maricao, as compared with Mayaguez, it was possible to grow some vegetables more successfully at Maricao, especially at the beginning and end of the winter season. During January, February, and March 1946 the best vegetable garden was grown at Maricao. This was attributed to moderate but not heavy rainfall and to the cool temperatures characteristic of the region during the late winter season. Vegetables which did well at Maricao during the winter season were: Georgia collard, Dwarf Blue kale, Wisconsin All Seasons cabbage, Danvers Half Long carrot, Broad-Leaved Batavian endive, Melting Sugar pea, Purple Top White Globe turnip, Logan bush bean, Chi Hi Li Chinese cabbage, Rhubarb Swiss chard, Puerto Rico No. 39 cucumber, Florida Broad Leaf mustard, and Cocozelle squash. Vegetables which gave fair yields were American Improved rutabaga, Calabrese broccoli, Hawaiian and Indian cauliflower, Early White Vienna kohlrabi, and New Zealand spinach.

During the summer season at Maricao those vegetables which gave fair yields consisted of carrot, collard, endive, pea, radish, and turnip, of the same varieties listed above. Vegetables which were poor to almost complete failures were Hollow Crown parsnip, broccoli, Improved Long Island Brussels sprouts, cauliflower, celery, kohlrabi, head lettuce, rutabaga, spinach, and cabbage.

The results obtained at Toro Negro were similar to those at Maricao. The soil at Toro Negro is a heavy Cialitos clay; the garden is located on about a 15-percent northern slope. Vegetables which did well during the winter season were pea, carrot, parsnip, endive, kale, radish, turnip, Brussels sprouts, kohlrabi, and cabbage, of the same varieties as those grown at Maricao. The best crops were pea, collard, radish, parsnip, carrot, kohlrabi, and Florida Broad Leaf mustard.

Difficulty was encountered in growing seedlings in the open seedbed at Toro Negro because of the beating rains. A special seedbed was prepared about 3 feet wide by 15 feet long and covered with glass window frames to divert the excessive rain. The soil was treated with formalin 10 days before the seed was planted. Excellent seedlings were grown in this bed as compared with a neighboring uncovered bed. On the basis of results obtained with open seedbeds at all three locations, some type of waterproof shelter is highly recommended for successful production of seedlings. Another practice to be encouraged is the transplanting of seedling

cole crops, peppers, eggplant, andtomato to 4- or 5-inch pots, and thence to the field after the plants have attained 6 to 8 inches in height. Definitely better growth and stands have been obtained in these trials by this potting system. Bamboo pots were found convenient and economical for this purpose.

Insects which continued to be damaging to the vegetables were the diamondback moth (*Plutella maculipennis* Curt.) on cole crops, leafhoppers on beans (*Empoasca fabalis* De Long), and various cutworms and the mole crickets or "changa" (*Scapteriscus vicinus* Scudd.) on all seedlings. Troublesome diseases were viruses, which were particularly destructive on pepper, squash, and tomato. Blight was very destructive on tomato, especially following and during excessively wet periods. Yellows of cabbage, broccoli, and kohlrabi was a problem during wet periods. Leaf blight on carrots caused occasional damage, but the plants usually survived and eventually produced a satisfactory crop.

TROPICAL FRUITS

By Edward P. Hume and Pedro Seguinot Robles

Cooperative avocado propagation program initiated.—In cooperation with the Puerto Rico Agricultural Co., 3,000 avocado seed of the West Indian variety were planted for rootstocks in August 1945. The seed were planted in sand and transplanted to 1-gallon cans containing a steam sterilized mixture of three-fourths Toa sandy loam and one-fourth manure by volume with sufficient lime to secure a pH of approximately 7.0.

By June 1946 approximately 1,000 trees grafted to promising varieties of particularly late- and off-season bearing types were ready for planting in commercial orchards to be managed by the agricultural company. This will give the station opportunity to study further the commercial possibilities of selected avocado varieties maintained by the station for several years at Guayanilla, P. R.

Behavior of late avocado varieties observed over a 2-year period.—Monthly inspections were made over a 2-year period to determine yield and other characteristics of off-season avocado varieties growing in a cooperative planting near Guayanilla. The planting is located on Aguilita stony clay at an elevation of approximately 300 feet. The soil is derived from limestone and peculiar to the semiarid section of the southwestern part of the island. Annual rainfall is approximately 50 inches mostly occurring in late summer and early fall. The planting site is well protected except from the prevailing easterly winds. Several difficulties have prevented good orchard management. The lack of a readily available water supply has made irrigation and spraying almost impossible. Continuous supervision has been economically unfeasible. The high price of off-season avocados greatly encouraged larceny. This resulted in some loss of data on yield and maturity dates of the fruit and made quality determinations difficult. The brittle nature of the avocado wood and the disregard of proper picking methods by those who stole the fruit resulted in many broken branches with a consequent increase in wood infection.

The most prevalent disease has symptoms similar to rusty blight (*Oleosporium* sp.) which causes dieback and defoliation. Scab, cercospora leaf spot, and anthracnose appear to be prevalent, although no pathological studies have been made to date. The leaves of some varieties are

subject to the attack of the sugarcane weevil root borer (*Diaprepes abbreviatus* L.) which resulted in a shot hole appearance or skeletonization of the leaves.

The best variety in this avocado collection is Winslowson, which is a hybrid between the Guatemalan and West Indian races. The trees are relatively small to medium sized which makes picking easy and permits closer spacing in the orchard. The large size fruit matures in January and is covered by a thin, bright-green skin; yield is moderate to heavy. The flesh is of excellent quality and almost fiberless, containing a loose seed which is not large in proportion to the size of the fruit. This variety is susceptible to dieback and a necrotic condition of leaf tips.

The second best variety is Las Mesas 122, the origin of which is obscure. It was propagated from a tree in an old planting at Las Mesas, and is believed to be a Guatemalan variety, possibly Nimlioh. The single medium-sized representative tree bears the largest fruits in the collection which are oval with a tough, dark-green skin; quality is excellent. It appears to be moderately resistant to disease.

The two best late spring varieties have been Panchoy and Itzamna. Both are well represented in the collection and show considerable variation in extent of disease attack. The trees are medium sized with more spread than the two previous varieties. Fruits of the Itzamna are susceptible to sunscald because they are carried well below the drooping branches on long stalks. The medium-sized fruit has a point of attachment about ½ inch to one side of the long axis of the fruit. The Panchoy fruits are not quite so desirable because of the high proportion of the total volume included in the thick rough skin and the presence of some weak fibers in the flesh. They both keep well into the spring and fill a gap in the harvesting season.

Other good varieties holding well on the trees are Dickinson, Manik, and Tumin. Collinson cannot be overlooked, for in spite of low productivity and lack of pollen, its quality is high and it has relative resistance to insects and diseases.

Under the conditions at Guayanilla the following varieties should receive further testing before being discarded: Gottfried, Kanan, Knight, and Lyon. Varieties not worthy of further trial are: Benik, Fuerte, Ishkal, Lamat, Mayapan, Puebla, and Tertoh.

PLANT INTRODUCTION AND PROPAGATION

PLANT INTRODUCTIONS

By Edward P. Hume and Ruben H. Freyre

Seeds and plants received from 20 countries.—The station received 279 plant accessions during the year. Particular emphasis was placed on the introduction of promising legume cover crops and ornamental vines. The station now has a collection of over 100 species and varieties of ornamental vines.

Plantings of lowland chilte (*Cnidoscolus* (*Jatropha*) *tepiquensis* (Cost. & Gall.) McVaugh) made good growth during the first year at the Guánica substation where the annual rainfall is about 20 inches. Chilte, a source of chicle, appears to be one of the most promising plants tried during the past 3 years at Guánica, where there is definite need for new economic crops. Mortality was less than 4 percent and new shoot growth

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varied from 5 to 25 inches with some plants attaining a height of 60 inches. The lowland chilte grew much better than the other species tried at Guánica. The plantings at Mayaguez, Maricao, and Toro Negro, where rainfall conditions are higher and temperature somewhat lower, were inferior.

Two lovegrasses (*Eragrostis lehmanniana* Nees and *E. chloromelas* Steud.), received through the Soil Conservation Service, survived an extremely dry period at the Guánica substation. These grasses have been successful on poor soil under dry conditions in the southwestern United States. Four new varieties of sesame or "ajonjoli" (*Sesamum orientale* L.) were received from Venezuela and are being tested for superiority to local strains. Production of sesame in Puerto Rico has been limited because of a leaf disease and other difficulties.

During September 1945, 188 new introductions were planted on the station property at Las Mesas. By June 1946 several species were making particularly good growth and when fertilized appeared to be adapted to the Nipe clay soil at an elevation of 600 feet. Among the better plants were: *Aganosma acuminata* (Roxb.) Don., *Alstonia scholaris* (L.) R. Br., *Aristolochia elegans* Mast., *Bauhinia pauletia* Pers., *Cassia excelsa* Schrad., *Cordia serrata* Juss., *Dombeya natalensis* Sond., *Markhamia hildebrandtii* (Baker) Sprague, *Mimosa bimucronata* (DC.) O. Ktze., *Tectona grandis* L.f., and *Zizyphus mauritiana* Lam. Palms in general made poor growth.

In February 1945 the first fruit was matured on an introduced nut tree, *Lecythis usitata* Miers. The urn-shaped fruit had a dehiscent cap and was 8 inches in diameter, 7 inches long, and weighed more than 5 pounds. Seed within the "pot" was fairly good eating and had a flavor resembling that of the Brazil nut.

PLANT DISTRIBUTIONS

By Edward P. Hume and Rubén H. Freyre

Seeds and plants were distributed to 36 countries.—Shipments to foreign countries consisted of 429 separate seed packages and propagating material totaling 883 pounds, and represented 237 species and varieties. A new seed-exchange list was distributed to 135 interested individuals and institutions throughout the tropical world. Local distribution of plants totaled 16,478 consisting chiefly of ornamentals.

PROPAGATION STUDIES

By Norman F. Childers, David G. White, and Aida G. Villafañe

Manila grass outstanding for tropical lawns.—Manila grass, *Zoysia matrella* (L.) Merr., was introduced into Puerto Rico by the station in 1936, and has been propagated here until it now covers about 5 acres of lawn. The grass has proven definitely superior to such lawngrasses as centipede (*Eremochloa ophiuroides* (Munro) Hack.), St. Augustine (*Stenotaphrum secundatum* (Walt.) Kuntze), Bermuda (*Cynodon dactylon* (L.) Pers.), Java (*Polytrias praemorsa* (Nees) Hack), and carpet (*Axonopus compressus* (Swartz) Beauv.). Manila grass is chiefly a tropical and subtropical grass, but it can be grown as far north as Connecticut. Blades are short, tough, narrow, numerous, pointed, and dark green. The important advantages of Manila grass are that it crowds out most weeds and other grasses, remains green during hot

dry weather, and appears to have no serious insect pests and diseases. The grass grows well under moderate shade in competition with the trees for water and nutrients. It also grows satisfactorily under trees of dense shade, such as the mango, provided the lower limbs are pruned to 10 feet or more above the ground. Flower stalks appear mostly during the winter months in Puerto Rico but being short they are not particularly objectionable and can be cut with a rotary lawn mower.

Well-established Manila grass forms a mat which feels like a thick rug under foot. The blades will endure considerable punishment from wheel or foot wear and for this reason the grass serves well on playgrounds and similar areas which receive considerable trampling. Unfortunately, this toughness makes the grass hard to cut with a hand lawn mower, but a rotary power mower cuts it easily.

Propagation is done entirely by sod blocks or sprigs. A Manila grass lawn can be established in Puerto Rico in 9 to 20 months, depending upon the soil fertility, the water supply, and the planting distance between the sod blocks. Although no cost figures are available for comparison with other grasses, it has been generally observed at this station that Manila grass lawns, once established, are economical to maintain because of relatively less need for mowing and weeding.

In a fertilizer trial (*29, p. 35*) better growth of *Zoysia matrella* was obtained with an application of nitrogen fertilizer than with potassium, phosphorus, lime, or manure extract. In April 1946 the entire *Zoysia* lawn at the station showed poor growth and yellowish color. On the basis of the above results, ammonium sulfate was applied to most of the lawn by means of a 30-inch hand-fertilizer-spreading machine. On one large area 70 by 200 feet, ammonium sulfate was applied in alternate 30-inch strips with check strips between. At the end of a week and after two moderate rains, a deep green color developed on the strips receiving nitrogen. In about 2 to 3 weeks the treated strips showed a marked increase in growth and a thickening of the sod, which was still evident several months later. There was apparently little lateral movement of the fertilizer since the treated and untreated strips were clearly defined.

FORAGE CROP STUDIES

By Edward P. Hume and Rubén H. Freyre

Napier grass superior to Napier-millet hybrids.—Napier grass, *Pennisetum purpurem* Schum., sometimes called Merker grass and known more commonly in Puerto Rico as elephant grass, was introduced into Puerto Rico by this station many years ago. Since that time it has become one of the principal forage grasses. Glen W. Burton of the Bureau of Plant Industry, Soils, and Agricultural Engineering, hybridized this grass with pearl millet, *P. glacum* (L.) R. Br., at Tifton, Ga., and sent five of the better F_1 strains to Puerto Rico for trial. They were planted in a replicated experiment for comparison with a selected strain of Napier grass. The north half of the plots was cut four times, in August and December 1945 and March and May 1946, while the south half was cut three times, in October 1945 and March and May 1946. The hybrids began to flower in September and continued flowering heavily regardless of height until vegetative growth was resumed in the spring. The Napier grass being less sensitive to photoperiodism did not flower until October and never when less than 6 feet high. Yields of the better hybrids exceeded the Napier grass during the summer by as much as 25 percent,

but at other seasons Napier grass was the heavier yielder by a greater percentage.

Napier grass culms were as thick or thicker than any of the hybrids and they remained succulent during the entire year. Hybrid culms had smaller girth and were definitely more brittle and woody during the shorter days of the year.

The hybrids reached a maximum culm height of 15 feet during late summer but during the shorter days there was relatively little vegetative growth whereas the Napier grass culms were a foot shorter in summer but as much as 4 feet taller during other seasons.

Both the hybrids and the Napier grass had a higher dry-matter content during the short-day winter season. The August cutting averaged for all grasses 22.8 percent moisture while the December cutting averaged 31.3 percent. The hybrids always had a higher dry-matter content with the greatest differences occurring during the short-day period of the year.

Three palatability trials were made using three to four dairy cows in a corral with free choice of fresh, chopped grasses. Napier grass was consistently consumed to a greater extent than any of the hybrids. Consumption of the most palatable hybrid was 12 percent less even when the total ration was limited. The chopped fodder from the hybrids produced during the winter was so poor that palatibility tests were discontinued.

The results secured with these hybrids do not warrant recommendation or further planting particularly because of the low yield and poor palatability in winter when forage is limited.

WEED CONTROL

By David G. White and Aida G. Villafañe

2,4-D only partly effective against nutgrass.—During the past year the station initiated a new research program on the control of tropical weeds. The results of the early experiments are presented. Field plots 5 feet wide and 10 feet long heavily infested with nutgrass (*Cyperus rotundus*) were sprayed with a commercial weed killer known as Dow A-510, containing 70 percent of 2,4-dichlorophenoxyacetic acid as the sodium salt. Each treatment was replicated at least twice and duplicate counts of dead (brown), affected (yellow), and uninjured tops were made 15 days after spraying, each count covering an area of 4 square feet within plots. Only the above-ground portions of nutgrass were examined critically. Symptoms of 2,4–D injury consisted of a slight yellowing of the blades 1 week after application followed by further yellowing and eventual death within 3 to 4 weeks. In the meantime, new blades emerged but the source of these blades was not investigated.

The effects of various concentrations of Dow A-510 on nutgrass are shown in table 6. All applications were made within a relatively short period. Although the lower concentrations of 0.050 and 0.100 percent caused considerable yellowing, only a few blades were dead after 15 days. Concentrations of 0.150 percent resulted in 52 to 54 percent kill and 0.20 percent concentration resulted in 63 to 66 percent kill within 15 days after application.

In other trials Grasselli "Spreader and Sticker" was added at the recommended rate of 4.5 ml. per gallon of solution without causing outstanding differences. Applications of 0.15 percent Dow A-510 made

TABLE 6.—*Effects of various concentrations of Dow A-510 (2,4-D) on nutgrass 15 days after application*[1]

Concentration[2]	Hour applied	Temperature	Nutgrass tops			Dead	
			Total	Not affected	Affected		
Percent	*a.m.*	*°C.*	*Number*	*Number*	*Number*	*Number*	*Percent*
0.05	9:15	25	82	16	66	0	0
	9:20	25	68	12	56	0	0
.10	9:45	26	54	0	54	0	0
	9:50	26	59	3	60	6	10
.15	10:45	27	67	4	27	36	54
	10:50	27	66	0	32	34	52
.20	10:20	28	84	2	29	53	63
	10:25	28	88	4	26	58	66
Check	....	....	104	104	0	0	0
	....	....	95	95	0	0	0

[1]No rainfall on day of application or previous day. Average height of nutgrass—5.4 inches.
[2]625-ml. solution used per plot.

at various hours from 8:00 a.m. to 3:00 p.m. on the same day did not result in marked differences in spite of the fact that the temperature gradually increased about 7° F. Three successive applications of 0.15 percent Dow A-510 at 2-week intervals resulted in an average kill of 92 percent of the tops 15 days after the last application. Doubling or tripling the volume of spray required to cover a plot did not result in consistent increases in percentage killed. Other results indicated that if rain occurred within 15 minutes after application of 2,4–D, effectiveness of the weed killer was not reduced. The application of 2,4–D on plants wet with dew or artificially wet resulted in higher kill than with dry plants.

Although application of 2,4–D killed nutgrass tops, the underground parts were not extensively affected as evidenced by the development of vigorous new tops within a month.

In addition to the above trials on nutgrass, 2,4–D was found effective in eradicating the following plants at concentrations of 0.15 to 0.20 percent: Dayflower, *Commelina longicaulis* Jacq.; caladium, *Caladium* sp.; Royal waterlily, *Victoria regia* Lindl.; and water-hyacinth, *Eichlornia crassipes* Solms. The water-hyacinth is a serious problem in some streams and drainage ditches in Puerto Rico. The dayflower is one of the worst pests in canefields.

ENTOMOLOGY AND ECONOMIC ZOOLOGY

INSECT PARASITES AND PREDATORS

By KENNETH A. BARTLETT

Seven shipments of sugarcane borer parasites received from Brazil.—Through the cooperation of the Bureau of Entomology and Plant Quarantine, Division of Foreign Parasite Introduction, South American Parasite Laboratory, seven shipments of parasites of the sugarcane borer (*Diatraea saccharalis* (F.)) were received from São Paulo, Brazil.

The shipments consisted of four species of parasites all in the pupal stage namely: *Apanteles* sp. probably A. *xanthopus, Ipobracon* sp. probably I. *amabilis, Pathenoleskia parkeri,* and *Paratheresia diatraeae*

(Bréthes). After mating, a total of 1,795 *P. diatraeae,* 18 *Pathenoleskia parkeri,* 63 *Metagonistylum minense,*[7] and 8 *Ipobracon* sp. were liberated at Central Eureka, Hormigueros, P. R.

At the request of the Puerto Rico Agricultural Co., two shipments of *Hambletonia pseudococcina* Comp., a parasite of the pineapple mealy bug (*Pseudococcus brevipes* (Ckll.)) were sent to Vieques, P. R., where this company has a large planting of pineapples. One shipment consisting of 725 puparia was made on January 17, and another of 900 on January 31. These parasites were collected at Lajas where *Hambletonia* has become well established from introductions from Hawaii made by the station several years previous.

DDT INVESTIGATIONS

By Harold K. Plank

DDT unsatisfactory in control of West Indian fruitfly on mangoes.—Final results of the experiment (*21*) with DDT emulsion showed little or no control of the West Indian fruitfly (*Anastrepha mombinpraeoptans* Seín) on mangoes. The data in table 7 show that the fruits on the treated trees of both the Totafari and Cambodiana varieties in the main orchard were generally more heavily attacked than those on the untreated, regardless of whether one or two applications of DDT had been made.

It was apparent that there was considerable migration of flies into both orchards and that, under the conditions in which the sprays were applied, the DDT formulation used was ineffective in preventing extensive infestation.

Table 7.—*Puparia of the West Indian fruitfly recovered from fruits dropping from mango trees sprayed March 22 and May 21, 1945, with DDT emulsion*

Variety, treatment, and date	Total fruits held	Puparia recovered per 100 fruits from—		
		Tree 1	Tree 2	Total
Main Orchard				
	Number	*Number*	*Number*	*Number*
Totafari, 1 application, March 22	677	299.7	336.4	316.2
Totafari, 2 applications, March and May 21	926	126.3	207.9	103.3
Totafari, untreated	1,004	134.5	163.5	142.9
Cambodiana, 1 application, March 22	1,608	46.2	14.1	32.6
Cambodiana, untreated	1,502	13.1	17.9	16.9
Seedling Orchard				
Cambodiana, 1 application, March 22	2,915	38.8	29.7	34.6
Cambodiana, untreated	3,800	39.7	60.2	38.3

DDT-controlled house ants.—A 5-percent solution of DDT in kerosene, forced into the nests with a household sprayer, was effective in controlling the crazy ant (*Paratrechina longicornia* (Latr.)). No activity of the ants was noted within a period of over 1 year after treatment. Neighboring nests treated with "Pyrin" household spray containing a small percentage of "Lethane" became active again about 3 weeks after

[7] Included with *P. diatraeae* puparia.

treatment. With the elimination of the crazy ant, other species began to increase in numbers, namely *Crematogaster steinheili* Forel, *Solenopsis geminata* Fabr. (fire ant), *Wasmannia auropunctata* Roger (little fire ant), and *Tapinoma melanocephalum* Fabr. In spite of the difficulty caused by the multiple-nesting habits of some of these, particularly the small, black-headed *Tapinoma,* one application of the above DDT solution in the nests kept them under control for at least 2 months. Painted on the lower 12 or 15 inches of the legs of tables, it protected the tops for a similar period. With due precaution against food contamination, the general use of such a solution in the home has indicated that this is the most effective insecticide yet employed here for reducing or eliminating ants as well as many other household insects, particularly flies, mosquitoes, and crickets.

MISCELLANEOUS INSECTS

By Harold K. Plank

Grassworms injured station lawns.—Early in June grassworm injury became apparent on grama grass (*Paspalum conjugatum* Berg.) and Java grass (*Polytrias praemorsa*) on the station grounds. The injury increased in some locations until these grasses were almost completely defoliated by the end of June. The situation was possibly aggravated by a drought which prevented rapid regeneration of the grasses.

The damage was due in part to the fall armyworm (*Laphygma frugiperda* (A. & S.)), but most of the injury was caused by slim greenish larvae about ½ inch long that lightly webbed together the lower leaves of the grass. These larvae were identified as *Psara phaeopteralis* Guenée. In Puerto Rico both species of insects have been known to attack grasses more or less severely, but recovery has usually been rapid without the use of insecticides (*33, pp. 423–465; 34, pp. 262–263*). In areas under observation there appeared to be about four times as many *Psara* larvae present as of *Laphygma,* and about half of each were full-grown and preparing to pupate.

Areas of lawn which contained mixed grasses usually exhibited differences in susceptibility. Grama and Java grass were generally completely defoliated, while nutgrass (*Cyperus rotundos*) and centipede grass (*Eremochloa ophiuroides*) were not attacked and Bermuda grass (*Cynodon dactylon*) was only slightly attacked. Manila grass (*Zoysia matrella*) showed relatively little evidence of leaf feeding in most areas, although some spots 4 and 5 feet in diameter showed considerable browning as a result of worm injury. In these areas, *Psara* larvae were abundant and fed upon the middle and lower parts of the stems, which caused the tops to wither or die and brown spots to develop. Small flocks of the black Puerto Rican grackle or "Mozambique" (*Holoquiscalus niger brachypterus* Cassin), which are always present on the station grounds, were observed foraging over the infested areas and feeding on fall armyworms. There was little effective reduction in population of either insect, especially where the worms were protected by deep sod.

RAT CONTROL

By Harold K. Plank

Imitation olive oil doubled the take of rat baits.—The addition of corn oil, raw linseed oil, and coconut oil to rat baits in Hawaii has been

found effective in increasing acceptance (*9, pp. 163-167*). Since poor acceptance has frequently been experienced here, a trial was made of soybean oil artificially colored and flavored to imitate olive oil. Teaspoonful baits in lightly waxed paper were prepared as follows: Grated fresh, mature coconut meat 2 parts, corn meal 3 parts, crushed dry beans 1 part, and precipitated barium carbonate 1 part (all by volume), thoroughly mixed dry and then moistened with sufficient milk from the coconut to cause the particles to cling together when pressed lightly. One-half of the baits were dipped in the oil and the other half left untreated. Early in the evening one bait of each kind was placed in 85 locations 5 to 10 feet apart in seven buildings. Upon examination 1 to 9 days later it was found that the oiled baits were preferred; 20 percent were carried away and presumably eaten and 32.9 percent were nibbled or only partly eaten, while 9.4 percent of the unoiled baits were taken and 31.8 percent were nibbled. In one location where there was particularly good cover, all the oiled baits were taken as compared with two-thirds of the unoiled.

BAMBOO PRODUCTION AND INDUSTRIALIZATION

PROPAGATION AND DISTRIBUTION

By David G. White and José B. Huyke

Bamboo plantings made on mountain watersheds.—A considerable quantity of bamboo culm stumps was planted in cooperation with the watershed protection program of the Insular Forest Service. The protection of both new and old watersheds to reduce the silting of hydroelectric dams has become a major problem in Puerto Rico. The new varieties of bamboo introduced by the station in recent years give good soil erosion protection (*28*) and also an opportunity for agricultural income. The following introduced species of bamboo offsets were planted above the new Cidra reservoir: *Bambusa tulda* Roxb., 2,185; *B. longispiculata* Gamble ex Brandis, 3,225; and *B. tuldoides* Munro, 1,000. A bamboo planting also was made in the Toro Negro forest above the Guineo Dam. Culm stumps were planted on the square at spacing distances of 15, 20, and 25 feet in 10 adjacent rows of each distance in order to obtain data on spacing in large-scale plantings.

During the year, 1,785 culm stumps also were distributed to other public agencies particularly through the efforts of the Soil Conservation Service, the Agricultural Extension Service, and the vocational agriculture schools. A total of 612 culm stumps were distributed directly to interested farmers. The development of a successful bamboo program in Puerto Rico has created considerable interest in this crop throughout the Caribbean area. A total of 193 bamboo offsets were sent to Dominica, Antigua, and Jamaica, B. W. I., for the development of new plantings.

Bamboo fishing rod industry established.—During the latter part of the fiscal year a new fishing rod industry, utilizing locally grown bamboo, was established near Mayaguez. The bamboo was supplied by the station through the Puerto Rico Development Co. and consisted mostly of *Bambusa tulda*. In addition, the use of newly introduced bamboos for miscellaneous novelty articles and for furniture has resulted in the establishment of two other new small industries in Puerto Rico.

In a report[8] recently released by the Army Quartermaster Corps it was revealed that tests made during the war on two bamboo species, *Bambusa tulda* and *B. tuldoides*, grown in Puerto Rico, had shown that the material was highly satisfactory for the manufacture of laminated bamboo ski poles.

The experimental bamboo shop supported by funds provided by the Insular Government was closed during the year in order to reduce Insular expenditures and also because all industrial phases of the bamboo program are now being largely handled and expanded under the auspices of the Puerto Rico Development Co.

SEED STORAGE

By David G. White and Aida G. Villafañe

Calcium chloride prolonged longevity of bamboo seed.—The flowering of *Bambusa arundinacea* Retz. in February and March 1945 offered an excellent opportunity to determine seed longevity under different storage conditions. A series of storage treatments were initiated as follows: Two lots of seed were placed in jars, one over hydrated lime and the other over calcium chloride at (1) room temperature (70° to 90° F.), (2) 60°, (3) 60° to 70°, and (4) 70° to 80°; other treatments were (5) check seed in jars at room temperature, and (6) over potassium pyrogallate in a desiccator at room temperature. The initial moisture content of the seed was 18.3 percent. Periodically, 100 seeds in each treatment were removed and germinated on filter paper over moist sphagnum moss in closed petri dishes under room conditions.

There was a definite decline in viability of bamboo seed over a period of 202 days in all treatments held at room temperature except those over calcium chloride; check-seed failed to germinate. The method of storing bamboo seed over calcium chloride at room temperature was the simplest and most practical; germination was 76 percent as compared with the best, 79 percent, for seed stored over calcium chloride at 50° F. Under conditions of this experiment, calcium chloride appeared to be definitely superior to hydrated lime for bamboo seed storage.

BAMBOO POWDER-POST BEETLE

By Harold K. Plank

Susceptibility of Bambusa tuldoides culms varied at different ages.—During the spring of 1945 a comparative study was made of the susceptibility of *B. vulgaris* Schard. and *B. tuldoides* to attack by the bamboo powder-post beetle (*Dinoderus minutus* (F.)). This test was similar to that performed in 1944 with *B. vulgaris* and *B. tulda* (*23*). Freshly harvested culms from 1 to 5 years old of each species were used. The test pieces from all five ages of *B. vulgaris* averaged 7.9 attacks each and those of *B. tuldoides* 1.2 each, a highly significant difference. Such a difference also existed between the two species in each growth year. First-year *B. tuldoides* in this test was only 7.7 percent as susceptible to the powder-post beetle as first-year *B. vulgaris*. The susceptibility of

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[8] McCLURE, F. C. WESTERN HEMISPHERE BAMBOOS AS SUBSTITUTES FOR ORIENTAL BAMBOOS FOR THE MANUFACTURE OF SKI POLE SHAFTS. Natl. Res. Council, Com. Quartermaster Prob., Final Rpt. QMC-24. 92 pp., illus. [1944.] [Processed.] Formerly restricted; obtainable in photostatic form from the U. S. Department of Agriculture Library, Bibliofilm Service.

the culms of *B. vulgaris* decreased successively with age, as in the previous test, except that the decrease was not significant between the fourth and fifth growth years. On the other hand, with *B. tuldoides* there was a slight increase in susceptibility up to the third year, and thereafter a decrease, but none of these differences was significant.

In both species there were more beetle attacks in the test rings taken from the bottom of the culms than in those from the middle, and more in the middle than in the top. These differences were highly significant in *Bambusa vulgaris,* but not in *B. tuldoides.* The usual iodine spot test indicated a concentration of starch corresponding to the severity of beetle infestation. Samples from *B. vulgaris* in general showed more starch than those from *B. tuldoides.* At any one location in the culms of either species there was little difference between ages in the amount of starch present, with the possible exception of the middle and top of *B. vulgaris,* both of which generally showed somewhat more starch in the young wood than in the old.

Bakelite-impregnated bamboo resistant to beetle attack.—In cooperation with the Puerto Rico Development Co. and the Charles F. Orvis Co., Manchester, Vt., pieces of *Bambusa tulda* and *B. vulgaris* in their second year of growth or older were impregnated with liquid bakelite under pressure. Upon solidification of the bakelite the character of the wood of both species was markedly changed; it had a phenol odor and a darker color and was heavier and considerably harder. However, the vessels in the fibrovascular bundles were still open and apparently suitable for oviposition by the powder-post beetle.

Several pieces of the bakelite-treated and untreated bamboo of both species were exposed to the bamboo powder-post beetle for 7 months in the laboratory, after which they were placed in paper bags with freshly emerged insects. None of the bakelite-impregnated pieces became infested. Among the untreated samples, those of *Bambusa tulda* were entered at only a few places, while those of *B. vulgaris* were heavily attacked. The bakelite treatment did not perceptibly change the starch in the wood; the treated samples reacted to the iodine test about as strongly as the untreated. Protection was evidently brought about by making the starch unattractive or unavailable to the beetle.

VANILLA

AGRONOMIC STUDIES

By Héctor R. Cibes and Norman F. Childers

Nitrogen and phosphorus deficiency restricted growth of vanilla.—In March 1945 an experiment was initiated to study the effects of deficiencies of nitrogen, potassium, phosphorus, magnesium, and calcium upon growth and fruiting of vanilla (*Vanilla fragrans* (Salisb.) Ames). The plants were grown in a shaded greenhouse in 5-gallon stone crocks filled with ¼-inch screened creek gravel. The crocks were equipped with 1-inch holes at the base for drainage. Each treatment was replicated three times. The Hoagland-Arnon solutions were changed once a month and pumped to the roots once a day. The pH was maintained at 6.0 by additions twice weekly of weak solutions of sulfuric acid or sodium hydroxide. The vanilla vines were supported on cured bamboo in order to minimize the possibility of nutrient materials being absorbed through the holdfasts of the vanilla.

After nine months, plants growing under nitrogen deficiency showed the most restricted growth. The plants were straw yellow and growth was only about 1¼ inches per plant per month. However, root development on the minus-nitrogen plants, although yellow in color, was relatively good and free from disease. This is in agreement with the characteristics of most other plants growing under nitrogen deficiency.

Phosphorus deficiency was second in importance to nitrogen deficiency in restricting growth. The minus-phosphorus plants closely resembled plants in the field dying, supposedly, from vanilla root rot (*Fusarium batatatis* var. *vanillae* Tucker). In most cases aerial roots on the phosphorus-deficient plants reached the gravel and died; roots beneath the gravel were scanty and over 50 percent dead and rotting. There was some evidence of root dying with minus-magnesium and minus-potassium plants also.

Plants deficient in potassium appeared to be a darker green than those receiving a full nutrient solution. Girth of the vines and leaf area were definitely smaller (about 15 percent less) than with plants receiving the full nutrient solution.

No nutrient deficiency symptoms appeared with the minus-calcium plants. These plants appeared as healthy as those growing in full nutrient solution, except for a moderate amount of root dying. A quick-chemical test was made to determine if the solution was absorbing small amounts of calcium from the creek gravel, but none was found after the solution had been pumped through the gravel for a month.

In conjunction with this experiment distilled water was pumped to three crocks containing mulch grown on Soller, Toa, and Catalina clays, respectively. Plants in all mulches grew as well or better than those grown in gravel supplied with full nutrient solution. In fact, plants grown in mulch from Soller clay had larger leaves, larger girth, and more shoot growth than those grown in the full nutrient solution.

Poor survival of vanilla at high and low pH.—Using the same method and procedure as described above, another experiment was initiated to study the effects of pH of the root medium on growth and survival of vanilla. The common dwarf bucare (*Erythrina berteroana* Urban) was employed as supports in place of bamboo. Two plants were grown in each crock, three crocks per treatment, at five pH's, namely 4.0, 5.0, 6.0, 7.0, and 8.0.

From the standpoint of growth and appearance of the plants as a whole, it was apparent that pH 6.0 was the most favorable hydrogen-ion concentration for vanilla, pH 5.0 next, and pH 4.0, 7.0, and 8.0 were the least favorable. Four plants died in the solution maintained at pH 7.0, two plants in pH 8.0, and one plant in pH 6.0. The dwarf bucare leaves showed yellowing and dropping at the lower pH valves which was to be expected of a leguminous plant.

Vanilla grown under lath shade showed promise.—In March 1944 an experiment was initiated (*6*) in which about one-third of an acre of vanilla was planted under a bamboo lath shade admitting two degrees of light. The vanilla is growing on dwarf bucare and has been mulched heavily with plant material consisting of grasses and weeds obtained from three types of soil: Toa, Catalina, and Soller. In half of the treatments the mulch was mixed with agricultural limestone.

After 18 months of growth, a third measurement of shoot growth was made. The data derived from this measurement are given in table 8. Analyses of variance of the data showed no significant differences among light treatments and no significant interaction between light and mulches. There were, however, significant differences between growth of vanilla in the three types of mulches, with or without limestone. Toa mulch without limestone produced significantly better growth than Catalina mulch alone or Soller, Toa, and Catalina mulch with limestone. The growth was not significantly better than that obtained with Soller mulch without limestone. Soller mulch without limestone produced significantly better growth than that obtained in Catalina mulch with or without limestone. Both Soller and Toa mulch with limestone produced significantly better growth than Catalina mulch with limestone. Up to this time, the Toa and Soller mulches without limestone have resulted in the best vegetative growth.

TABLE 8.—*Shoot growth of vanilla under two degrees of light and growing in mulches obtained from three different soils, mixed with and without limestone*

Mulch treatments	Shoot growth[1]		Total shoot growth per mulch treatment[2]
	½ roof area covered	⅓ roof area covered	
	Inches	*Inches*	*Inches*
Catalina	1,204	1,083	2,287
Toa	1,272	1,350	2,622
Soller	1,264	1,272	2,536
Catalina and limestone	1,081	1,146	2,227
Toa and limestone	1,171	1,249	2,420
Soller and limestone	1,221	1,213	2,436

[1] Total shoot growth made on 8 separate plots for each mulch treatment, 4 plants per plot.
[2] Difference necessary for significance at 5 percent level—172.69.

Because of the heavy intertwined growth of the vines by March 1945, it was impossible to accurately take a fourth measurement. Data hereafter will be confined to yields and quality of beans which, in the final analyses, are of most interest to the grower. Excellent growth was obtained up to July 1946, 2 years and 3 months after planting. The amount of vegetative growth was estimated to be equivalent to the average growth obtained in a 5- or 6-year-old planting growing under the best field conditions. Girth of shoots was about ¾ inch, which is considerably larger than the average for vines grown under field conditions. During 1946 a total of 56 vines flowered out of 393 plants in the experiment and the beans produced were extra large in size.

Although this planting is showing exceptionally good growth at the end of 27 months, 2 years or more are needed before its economic possibilities can be estimated. The good growth to date is attributed to (1) uniform shade throughout the year, (2) a heavy mulch of 10 to 12 inches maintained continually, and (3) irrigation during periods of extended dry weather.

CHEMISTRY OF VANILLA PROCESSING

By Gilda C. Vicente and Merriam A. Jones

High percentage of vanillin formed within 12 days after killing.—In the 1945 annual report (*26*) it was shown that important enzymatic

changes in vanilla beans occurred within the first 2 weeks after the beans were killed. In that experiment the enzyme systems were inactivated by autoclaving samples of beans at 120° C. at various stages during the curing process. Additional data, including data on vanillin analysis of these beans, were obtained during the past year. The accompanying tabulation shows the vanillin content of the beans at different stages in the curing process.

TABLE 9.—*Vanillin content of vanilla beans at different stages of curing*

Stage of curing	Vanillin content
	Percent
Immediately after killing	0.31
2 days after killing	.46
12 days after killing	1.54
42 days after killing	1.99
Controls (completely cured)	2.44

Ground vanilla cured to a high vanillin content.—In the last report (*26*) an experiment was described in which the beans were cut to different degrees of fineness. Beans cured whole contained 3.5 percent of vanillin, those cut to 1-cm, slices, 3.21 percent, those ground in the food chopper, 3.65 percent, and those ground with sand, 3.79 percent. Although the differences in vanillin content were relatively small they were consistent and presumably due to the treatments. Apparently, the sliced beans lost some vanillin by sublimation while the ground beans had even more vanillin than the controls because of the intimate contact brought about between the glucovanillin and the hydrolizing enzyme and because of the decreased exposure. Organoleptic tests in ice cream made with these extracts indicated that no differences existed among these four treatments. Because of the simplicity of curing ground vanilla, these processes appear promising but further testing will be necessary before they can be definitely recommended.

Outer wall of vanilla bean had highest vanillin content.—The seed and placental tissue of vanilla beans were cured separately from the pod wall and extracts were prepared from these portions for analyses. The seed and placental tissue contained 2 percent vanillin on a dry basis, but upon incubation of the extract with emulsion the percentage increased to 2.74. It can be assumed from these results that glucovanillin was present in the extract. The cured outer wall contained 4.03 percent of vanillin. Calculations based on the original and final weights and moisture contents showed that the outer wall constituted 64.7 percent of the dry matter of the beans and 72.8 percent of the vanillin. The seed and placental tissue contained 35.3 percent of the dry matter and 27.2 percent of the vanillin. Extract from the outer wall was dark and flavorful while extracts from the seed and placental tissues were light colored and weak in aroma. As in previous work (*1, pp. 11-14*), it was concluded that the outer portion of the vanilla bean was responsible for most of the flavor.

Killing beans by hot water or freezing gave best results.—In a previous study (*26*), beans were killed by several methods as listed in table 10. The results of vanillin analyses of the extracts from these beans are presented in the following tabulation.

TABLE 10.—*Vanillin analyses of vanilla beans killed by different methods (dry basis)*

Killing method	Vanillin content
	Percent
Hot water	2.80
Scratched	3.30
Frozen	2.86
Hot water—scratched	2.85
Scratched—hot water	2.96
Hot water—frozen	3.00
Frozen—hot water	2.66
Scratched—frozen	2.86
Frozen—scratched	1.93

From these data it is apparent that beans killed by a combination of two curing methods were no better than beans killed by any single method insofar as vanillin content was concerned. Scratching the beans resulted in the highest vanillin content, as previously noted (*26*). However, organoleptic tests with ice cream containing the extract indicated that there was no detectable difference in flavor. It was found that the extract made from beans killed by hot water usually scored highest, with those killed by freezing second best, but the difference between the two was not statistically significant. However, both were significantly superior to the extract from beans killed by scratching, and the latter method was significantly better than the blank. The scratched beans appeared to be better because of the rapid rate of curing, vanillin crystallization, high vanillin content, and good aroma. Beans killed by hot water and freezing methods, however, produced the strongest and best extract as measured by the ice cream test. These beans did not have the highest vanillin content among the treatments. This indicates that there are substances other than vanillin that contribute to the aromatic and flavoring properties of vanilla beans.

Hot water killing and conditioning at 45° C. was superior.—It has been shown that conditioning above 27° resulted in a superior product (*26*). Beans conditioned at 45° and 35° had a strong vanilla aroma accompanied by a characteristic prunelike odor. The prepared extracts were filtered through paper to remove fine solids and it was noted that the vanilla conditioned at the higher temperatures filtered more rapidly than that conditioned at the lower temperatures. This indicated that finer solid particles were probably suspended in the extracts of the beans conditioned at lower temperatures.

The vanillin content for the beans conditioned at 45° C. was 2.07 percent, at 35°, 2.21 percent, at 27°, 2.55 percent, and at 13°, 2.73 percent. The corresponding moisture contents for these beans were 14.1, 18.3, 20.5, and 18.5 percent, respectively. Organoleptic tests of the extracts in ice cream showed that the extract from beans conditioned at 45° was significantly superior to the others. Beans conditioned at 35° were significantly better than beans conditioned at room or refrigerator temperatures. Of the latter two, the beans conditioned at room temperature scored higher than beans conditioned at 13°, but the difference was not statistically significant. It was concluded from these results that conditioning vanilla beans at 45° was best.

Beans harvested early in the season gave a superior cured product.—An experiment was initiated to determine the quality of vanilla beans picked at different times during the harvesting season. For the purpose of this experiment three harvesting dates were selected: (1) Early season (November 3); (2) midseason (December 27); and (3) late season (January 31). In order to insure a uniform degree of maturity, all beans were harvested at the blossom-end-yellow stage from the station plantings.

Beans harvested early in the season were rather small. They measured 4½ to 7½ inches in length, the majority of which were about 5½ inches. Moisture content of beans at this stage of maturity was 80.07 percent. During the sweating and drying phases of the curing process no unusual characteristic was noted in this group. In fact, the beans had developed a strong vanilla aroma which was considered very good. After conditioning at 45°, the vanilla odor became stronger, and the beans appeared somewhat oily and black in color. The vanillin content was 2.67 percent, but no vanillin crystallized. In general, they had qualities similar to Mexican vanilla.

Beans harvested during the middle of the season were from 8 to 9½ inches in length. The moisture content of these beans averaged 79.48 percent. During the sweating and drying phases they developed a slight vanilla aroma accompanied by a sweetish note. This aroma lacked strength and was definitely inferior to that of beans harvested early in the season. During the conditioning process the aroma was improved somewhat, although the usual character of beans conditioned at 45° was never developed. The beans were shiny and oily, which gave them a pleasing appearance, but they were somewhat light in color. The vanillin content was 3.59 percent, but no vanillin crystallized.

The beans harvested at the end of the season measured from 8 to 10 inches in length and contained 79.66 percent moisture. During the sweating and drying phases they developed a bouquet similar to that of the beans gathered at the midharvest period, which improved with further conditioning. Although there was profuse vanillin crystallization, there was little difference in the character of the bouquet and general appearance of these beans and those harvested in midseason. The vanillin content was 4.17 percent.

As recommended by Gnadinger (*11, p. 29*), a 5-percent solution of the extract in water was made to compare the flavor of the three extracts. The flavor of extract of beans harvested early in the season was stronger than that of beans harvested in midseason and late season.

Fifty milliliter of milk flavored with 1 ml. of the extract was also tested and the results obtained were similar to those tested with water. On the basis of these experiments it appears that beans harvested early in the season have the best aromatic and flavoring qualities although they are usually somewhat smaller and have a lower vanillin content than beans harvested in midseason or late season.

Ice cream tests with these samples will be made before final judgment is passed.

Blossom-end-yellow beans gave the best vanilla extract.—In a previous experiment (*19*) it was noted that blossom-end-yellow beans developed an aroma superior to that obtained from green beans. In the same experiment in which beans were harvested when completely brown,

it was found that they had a higher vanillin content than green beans, but a lower content than beans harvested at the blossom-end-yellow stage.

An experiment was initiated to study the effect of maturity at the time of harvest on vanilla quality. For this purpose beans were harvested at several stages of maturity as follows: (1) Entirely green, (2) blossom-end-yellow stage, (3) blossom-end-brown stage, and (4) with beans entirely chocolate brown.

Moisture contents of beans harvested at various stages of maturity were as follows: Green beans 82.14 percent, blossom-end-yellow beans 80.47 percent, blossom-end-brown beans 67.05 percent, and entirely brown beans 59.07 percent. It was apparent that as the degree of maturity advanced the moisture content decreased.

During the sweating period green beans developed a slightly fermented aroma which disappeared during the drying process. Vanilla aroma was faint, although by the end of the conditioning period it improved to some extent and had a sweetish tinge. However, it never attained the true vanilla odor characteristic of beans of high quality. The beans were dull light-brown in color and had a gummy texture. It is evident from these results that vanilla beans should not be picked at this stage of maturity since they produce an undesirable cured product which brings a low market price.

Beans harvested at the blossom-end-yellow stage gave a good cured product. They possessed a dark-brown color, were oily, and had a pleasing vanilla bouquet.

Beans harvested at the blossom-end-brown stage developed a fruity aroma which was considered agreeable but definitely lacked true vanilla character. Later, the aroma became fragrant and similar to that of beans killed by scratching. The majority of the beans were split and there was good vanillin-crystal formation.

Beans which had become completely brown while attached to the vine developed an aroma after curing similar to that of blossom-end-brown beans although it was not as pronounced. There was a profuse vanillin crystallization.

Quality of beans not influenced by altitude of vanillery.—Among the factors that may influence the quality of vanilla beans is the altitude at which a vanillery is located. Since temperature is known to affect the time of fruit set, it is possible that it may influence the appearance and quality of the beans. An experiment was conducted in which quality of vanilla beans from different altitudes was analyzed. The beans were harvested from vanilleries at the following locations: Mayaguez (80 feet), Las Mesas (600 feet), Morovis (700 feet), and Castañer (1,800 feet). All beans were collected with the blossom-end-yellow and cured by killing in hot water and conditioning at 45° C.

At the time of harvest, beans from Mayaguez contained 77.07 percent moisture; beans from Las Mesas, 81.26; beans from Morovis, 81.88; and beans from Castañer 78.82 percent.

During the curing process marked differences in aroma were noted. Beans from Mayaguez developed a strong vanilla aroma during the sweating phase which persisted throughout the curing. They were oily, shiny, and dark in color. During conditioning at 45°, a temperature which has produced good results, these samples acquired a good aroma and appearance.

Beans from Las Mesas appeared to be the best green vanilla of all samples taken, but they did not develop a true vanilla aroma. The character was faint and there was a sweetish tinge in the aroma. They were somewhat oily and lighter in color than beans from Mayaguez.

Beans obtained from Morovis developed an appearance and aroma characteristic of vanilla harvested at the green stage. They were dull and light brown in color, not oily, and the texture rather gummy. They developed only a slight vanilla aroma and were considered of poor quality. Vanillin did not crystallize.

Samples from Castañer developed an oily and suave vanilla character and a sweet tinge during the sweating process. There was also a foreign, somewhat flowery odor present. Beans conditioned at 45° developed a good vanilla aroma but to a lesser degree than beans from Mayaguez. Vanillin did not crystallize in any of the samples.

Extract made from vanilla grown at Mayaguez was the best, followed in order by samples from Castañer, Las Mesas, and Morovis.

From these results it appears that altitude itself does not appreciably influence quality of cured beans, inasmuch as beans from an altitude of 80 feet above sea level had as good quality as those from an altitude of 2,000 feet. The difference in quality of beans and extract among the various samples must be attributed to factors other than altitude, as, for example, soil or rainfall conditions.

No difference in quality between beans from healthy and diseased plants.—The quality of vanilla beans cured from plants having vanilla root rot disease was compared with that of beans from healthy plants.

Samples of beans were collected from eight healthy and eight diseased plants. The beans obtained from the healthy plants averaged 7½ to 9 inches in length while those picked from the diseased plants averaged only 5 to 7 inches. Moisture determination of the beans at harvest showed that those obtained from healthy plants contained 78.64 percent and those from infected plants 80.22 percent. Both lots of beans were cured by killing in hot water and conditioning at 45° C. and during the process no differences in aroma were noticed. Both had a good vanilla character. After conditioning, beans from healthy plants contained 33.38 percent moisture and those from diseased plants, 36.56 percent. There was no vanillin crystallization or mold development in either of the samples. Beans from healthy plants contained 3.45 percent vanillin while those from diseased plants had 2.97 percent. The extracts were tested in water solution and milk and no differences in flavoring properties could be detected. It is concluded that the disease had little if any effect on the quality of the cured beans.

ESSENTIAL OILS

AGRONOMIC STUDIES

By Pedro Seguinot Robles and Noemí G. Arrillaga

Lemon grass cut at 2½ feet yielded maximum oil per acre.—Additional data have been accumulated on the height-at-harvest experiment for lemon grass (*Cymbopogon citratus*) (DC.) Stapf.). (*25, pp. 53-54*). The results obtained over the first 32 months are given in table 11.

It is evident from these data that the Java type was somewhat superior to the West Indian variety. The results to date showed that the greatest oil and citral returns per acre were obtained when the grass was cut at 2½ feet. However, as would be expected, more oil and citral per individual harvest were obtained when the grass was cut at maximum height. Therefore, it becomes a question of economics whether the grass should be cut more frequently at 2½ feet or less often at maximum height.

TABLE 11.—*Yields per acre of grass, oil, and citral from lemon grass cut at three heights over a period of 32 months*

Variety	Height of harvest	Harvests	Yields per acre		
			Grass	Oil	Citral
	Feet	*Number*	*Pounds*	*Pounds*	*Pounds*
Java	2	15	85,163	231	179
	2½	14	108,843	360	276
	3 to 3½	8	117,956	326	247
West Indian	2	15	81,174	265	197
	2½	14	89,436	332	256
	3 to 3½	8	91,576	283	215

Frequent cutting of lemon grass caused high mortality.—The cutting of lemon grass at 2 feet in the "height-at-harvest" experiment described above was too severe for the best development of the grass. When cut at this height, mortality in the West Indian variety averaged 32.5 percent and for the Java 17.5 percent. Mortality of the grass when it was cut at 2½ feet was the same for both varieties, 14 percent. When the grass was harvested at its maximum height, mortality was reduced to 8.0 percent for the West Indian and 3.0 percent for the Java varieties.

Rate of growth of lemon grass influenced by rainfall.—Climatic factors such as temperature and rainfall may affect growth and oil content of lemon grass. To obtain information on these points the yields of grass obtained in the height-at-harvest experiment previously discussed were correlated with temperature and rainfall records during the experimental period.

The data obtained indicated that growth of the grasses followed a definite cycle. When the yields of the first harvest were taken as normal and compared to the yields of subsequent harvests, it was apparent that the grass had gone through a period of rapid increase which coincided with the beginning of the rainy season. With the cessation of rains a progressive decrease in the yields of grass resulted. During the driest period the yields were lowest and tended to remain low until the onset of the rainy season. The Java variety seemed to be more affected by variations in rainfall, resulting in a rapid increase in yields of grass with heavy rains and a rapid decrease when soil moisture was low. During periods of heavy rain the grass tended to grow faster but did not stool as well. This resulted in a shorter interval between harvest with lower yields of grass.

The average temperature between each of the harvests varied little. Growth of grass was more directly affected by the wide variations in rainfall than by the small temperature differences.

Half of the lemon grass oil in the lower third of the clump.—In harvesting the lemon grass, the plants are generally cut 3 inches from the ground. Hood (*14, pp. 4-5*) found that the upper third of the grass gave 0.40 percent of oil containing 70 percent citral, the middle third 0.24 percent of oil containing 78 percent citral and the lower third 0.10 percent of oil containing 82 percent citral. This indicated that the lower portion of the grass is low in oil. However, the yield of grass was not taken into consideration.

A similar experiment was conducted in which the weights of the three sections of the grass were recorded. A large sample of lemon grass was cut about 3 inches from the base of the clump. The length of the leaves varied from 3 to 4 feet. The entire sample was then divided into three portions—lower third, middle, and upper.

It can be seen from table 12 that, although the percentage of oil was less in the lower part, the weight of grass was 66.3 percent of the total, and, therefore, the yield of oil per 100 pounds of whole grass was the highest. The citral content was highest in the lower third of the grass. These data showed that about one-half of the oil and citral was in the lower third of the grass.

TABLE 12.—*Comparison of the yield of grass, oil, and citral from different parts of the lemon grass plant*

Part of leaves	Weight of grass	Fraction of total grass	Yield of oil	Yield of oil per 100 pounds of entire grass	Citral	Yield of citral per 100 pounds of grass
	Pounds	*Percent*	*Percent*	*Pounds*	*Percent*	*Pounds*
Top	40.0	11.3	0.533	0.063	78	0.049
Middle	74.0	21.9	.305	.067	82	.055
Lower	224.5	66.3	.179	.119	86	.102
Total	338.5	100.0	.249	.249	82.7	.206

Harvesting citronella grass at 4½ feet yielded more oil per acre.—A height-at-harvest experiment similar to the foregoing was started in 1943 with the Guatemala and Java varieties of citronella grass (*Cymbopogon nardus* (L.) Rendle). The main difference in the two experiments was that the citronella grass was cut at 3½ feet, 4½ feet, and maximum height of about 6 feet. A preliminary report on the experiment was made in 1945 (*25, p. 54*). Additional data have been obtained on the yield of grass, oil, and total geraniol per acre under the three treatments. The results are summarized in table 13.

Both varieties of citronella grass yielded approximately the same amount of grass and oil during the experimental period confirming the prevailing belief that both grasses have the same botanical origin.

It is evident from the data in table 13 that cutting the grass at a height of 4½ feet yielded more oil and geraniol per acre than when it was cut at 3½ feet or at maximum height. To fully evaluate the practical aspects of this experiment it is necessary to take into consideration the added cost of production incurred by more frequent harvests when the grass is cut at 4½ feet instead of at 6 feet.

TABLE 13.—*Yields per acre of grass, oil, and total geraniol from citronella grass cut at three heights over a period of 3 years*

Variety	Height at harvest	Harvests	Yields per acre		
			Grass	Oil	Total geraniol[1]
	Feet	*Number*	*Pounds*	*Pounds*	*Pounds*
	3½	10	61,028	324	195
Java	4½	8	84,867	471	293
	6	5	82,832	346	208
	3½	10	57,377	311	185
Guatemala	4½	8	71,769	452	268
	6	5	79,615	374	236

[1] Determined as acetylizable constituents.

As was the case with lemon grass, considerable mortality resulted when the grass was allowed to grow to its maximum height, the tufts became dense, turned yellow, and tended to die out.

Analyses of bay oil from different districts continued.—The analysis of 24 additional samples from bay rum trees (*Pimenta racemosa* (Mill.) J. W. Moore) located in the districts of Guayama and Ponce brought the total number of analyses from Puerto Rico and the U. S. Virgin Islands to 120 (*25, pp. 54-55*). The samples from Guayama showed but little variation in the mean oil content when compared to samples previously analyzed from this district. The samples from Ponce showed a definite increase of 0.40 percent in the mean oil content over the previous analyses. This may be due to the fact that 2 months before the samples were taken, the grower had fertilized his bay rum trees with ammonium sulfate at the rate of 1 pound per tree.

Ten samples were analyzed from three localities on the Island of St. John, U. S. V. I. The oil content of these samples varied considerably with the location. Those from Hognest Bay yielded the highest oil percentage of any of the samples analyzed to date. The samples from the other two areas, Cinnamon Bay and Dennis Bay, were of average oil content. The mean oil yield of these Virgin Island samples was 2.4. This mean is higher than any of the means from areas in Puerto Rico surveyed to date.

In all cases there was slightly more oil in leaves of the upper half of the tree compared to that of the lower half. This was probably caused by the difference in the age of the leaves. Similar trends were obtained with samples from Puerto Rico which showed that young leaves contain more oil than older ones.

The samples analyzed to date show that rainfall is apparently an important climatic factor influencing the growth of bay rum trees and the production of bay oil; oil content seems to vary inversely with the amount of rainfall. It is of interest to compare the yield of bay oil (2.4 percent) and annual rainfall (44.7 inches) at St. John with that of Cabo Rojo (2.1 percent oil and 49 inches of rain). Among the areas studied in Puerto Rico the Cabo Rojo area has the lowest annual rainfall and it produces bay leaves with the highest oil content. The latest samples analyzed from the Ponce district indicate that fertilization may also tend to increase the oil content. While high percentage of oil in the leaves

is desirable, the factor of most interest to growers is high production of oil per acre over a given period of time. A tree having high percentage of oil may not necessarily produce well on an acre basis. Trees with high oil content will be propagated on a larger scale at the station to determine their productive capacity on an acre basis.

PROCESSING STUDIES

By Noemí G. Arrillaga and Merriam A. Jones

Intermittent distillation of lemon grass gave good results.—In the steam distillation of essential oils from plant material, two processes occur. First, the volatile oil is liberated from the plant tissues, and second, it is volatilized with the steam and passes to the condenser. Whichever of these processes is the slower determines the over-all rate of distillation of oil. The first process can be hastened by such measures as cutting or bruising the plant material; the second by manipulating the boiler and stillpot pressure to increase the ratio of oil to water. In essential oil distillation the rate of liberation generally is the controlling factor. When free oil is steam distilled, the rate is very high compared to the rate of distillation of the same oil from plant material. Therefore, although high entrainment rates should be obtained by applying the principles of steam distillation, such rates cannot be obtained in practice because of the manner in which the oil is held by the plant tissue.

Although cutting the plant material or adding salt to the stillpot increases the rate of liberation to some extent, these practices are not sufficiently effective to free the oil rapidly. An alternative method of freeing the oil which has been recently tested consists of pressure cooking the plant material at intervals during distillation. By this method, it was expected that the first gush of oil, loosely held by the plant tissue, would distill over and then, as the rate of liberation again became limiting, the distillation would be stopped until more oil was cooked out. This oil would then be rapidly distilled as free oil and the process repeated intermittently.

To test this method 10-pound samples of chopped lemon grass were distilled by two methods. In the first method, distillations by the ordinary method were carried out as follows: The grass was placed in the still and steam applied for 5 minutes with the outlet valve to the condenser closed. The pressure in the stillpot rose to about 5 pounds per square inch. After 5 minutes the condenser valve was opened, and the distillation continued for 40 minutes longer. The total yields for two runs were 9.32 and 8.64 gm. of oil containing 7.76 and 7.20 gm. of citral. In the second method, intermittent distillation was the same for the first 10 minutes, at the expiration of which time the receiver was changed and the condenser valve closed. After 5 minutes, during which about 5 pounds pressure built up, the condenser valve was opened and distillation resumed for 10 minutes, when the valve was closed and the receiver changed again. After 5 minutes the valve was opened, and distillation resumed for 15 minutes. The total yields were 10.80 and 10.04 gm. of oil containing 8.35 and 7.82 gm. of citral. The yields by fractions showed that about half of the oil came over during the first period. However, the oil coming over during the second period was so much richer in citral that actually more citral was present in the second fraction. About 11 liters of water were distilled by the ordinary method and about 8 by the intermittent method.

This work was repeated by taking the yields by both methods in fractions. The ordinary method was as described in the foregoing experiment except that the receiver was changed every 10 minutes. The intermittent method was the same for the first 10 minutes when the condenser valve was closed for 5 minutes of each 10-minute period. During this time pressure built up and the oil was distilled in gushes. The distillations were carried out for 1 hour so that six fractions were obtained by each method. In both methods of distillation the oil was either extracted from the water and the products measured. Composite oil samples were analyzed for citral.

The results for two pairs of distillations are shown in table 14. The yields by 10-minute fractions are shown as percentages, the total yield by the ordinary method being assumed as 100 percent.

TABLE 14.—*Ordinary distillation of lemon grass compared with intermittent distillation*

Distillation	Yields by fractions							Citral in total oil
	1	2	3	4	5	6	Total	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*		*Percent*
Ordinary.........	18.0	22.9	38.9	7.1	7.6	5.5	100	
	16.2	32.5	22.6	11.4	11.9	5.4	100	79
Intermittent.......	18.3	43.5	27.9	10.9	10.3	5.0	115.9	
	17.9	22.5	26.9	22.1	13.6	7.1	110.1	84

It can be seen that the intermittent method was superior to the ordinary method. Most important was the fact that the distillation was more complete so that the total yield was 10 to 15 percent greater. Also important was the fact that the citral percentage was much higher in the oil distilled by the intermittent method. With intermittent distillation there was a saving in steam because distillation only occurred half as long; less water was distilled to obtain more oil.

Yield and quality of bay oil affected by storage treatment of leaves.—Among bay growers there is considerable variation in the treatment of bay leaves before they undergo distillation. Some leaves may be distilled almost immediately after harvest whereas others may not be distilled for several days or weeks. In connection with the discussion of a previous experiment (*2*) it was shown that bay leaves stored 8 days before distillation yield more and better quality oil than leaves stored only 1 or 5 days after harvest. In another trial progressively higher yields of bay oil were obtained up to 14 days of storage.

The treatments in the present experiment were as follows: (1) Bay leaves were placed in the refrigerator at 10° C. in paper bags; (2) kept at room temperature about 27° in closed and open bags; or (3) placed in the oven glass jars at 35°. Samples from each temperature treatment were withdrawn at intervals of 0, 1, 3, 6, 10, 15, and 21 days, respectively, and distillations were made according to standard procedure.

In most samples the yield of oil and phenols increased with the length of storage period, reaching a maximum after 7 to 10 days in storage. However, there was a decrease in oil and phenol content in leaves stored about 15 days, followed by an increase to the maximum content after a storage period of 21 days.

These results were confirmed in another similar experiment in which all leaves were stored at room temperature either in open or closed bags for periods ranging from 1 day to 4 weeks.

As in the first experiment the yield of oil and phenol increased with time of storage, reaching a maximum at 8 days, after which the amounts of both constituents decreased and remained at more or less the same level up to 21 days of storage. The leaves stored in the open bags gave somewhat better yields, probably because of the better circulation of air and more uniform drying, all of which may contribute to better preservation of the leaves. Both lots of treated leaves remained in good condition during the 4 weeks in storage. No mold developed and the oil obtained had a good aroma.

It is concluded that under the conditions of these experiments higher yields of oil and phenols can be obtained if the bay leaves are distilled after storing from 7 to 9 days. For experimental purposes it can be assumed that little or no change should occur in the oil and phenol content of bay leaves if they are kept in a refrigerator for 3 to 5 days before distillation. For commercial purposes bay leaves can be stored for a month but with little loss in oil and phenol content, provided they are kept under shade and are stirred occasionally for aeration.

SPICES

AGRONOMIC STUDIES

By Pedro Seguinot Robles

The use of manure favored yield of green ginger.—An experiment was carried out to test the effect of seven fertilizer levels on the growth of Chinese ginger (*Zingiber officinale* Roscoe). In the experiment, designed as a Latin square, the treatments were: (1) 15 tons per acre of leaf mold; (2) 15 tons per acre of barnyard manure; (3) 900 pounds of 6–9–10 commercial fertilizer per acre in split applications—450 pounds per acre at planting, and 450 pounds 3 months after planting; (4) 5 tons of leaf mold supplemented with 600 pounds per acre of 6–9–10 commercial fertilizer; (5) 5 tons per acre of barnyard manure supplemented with 600 pounds per acre of 6–9–10 commercial fertilizer; (6) 5 tons of a compost of equal parts of barnyard manure and leaf mold supplemented with 450 pounds per acre of commercial fertilizer 6–9–10; and (7) control with no treatment.

Definite variations were observed among the treatments in the amount of foliage developed. Foliage was obviously better where manure alone was applied and the control made the least vegetative growth. Some wireworm injury to the roots was noted in all treatments.

The yields of green root when analyzed for variance were not statistically significant except between the control and the plots receiving 15 tons per acre of barnyard manure. The manured plots yielded 14,848 pounds of green ginger per acre and the control 11,294 pounds.

The experiment is being repeated in the same plots under the same treatments over a period of several years in order to study residual effects. For the first crop there appeared to be no advantage in including commercial fertilizers.

Spice grove developing satisfactorily.—The experimental spice grove formerly occupying an area of 5 acres has been progressively in-

creased to 8 acres. This planting of spice crops is growing on land formerly devoted to coffee in an attempt to find new crops and increase the returns from coffee lands. A systematic planting program has been followed to grow nutmeg (*Myristica fragrans* Houtt.), cloves (*Eugenia caryophyllata* Thunb.), allspice (*Pimenta officinalis* Lindl.), and black pepper (*Piper nigrum* Linn.) within the established coffee plantings. These spices are planted among the shade trees, and in some instances shade trees have been removed to provide the necessary space and to reduce competition. Younger shade trees have been utilized as living supports for the pepper vines.

At this time the planting includes over 100 each of bearing and non-bearing nutmeg trees. Cinnamon is being grown on the edge of the planting and is being propagated rapidly. Allspice and cloves are making excellent growth within the planting. Only a few of the pepper vines are bearing, and these are being propagated to increase the plantings.

MISCELLANEOUS

By David G. White

A hood to protect precision instruments.—In tropical regions precision instruments, such as a microscope, require protection from humid atmospheres which are conducive to corrosion and to the development of a glass-etching fungus. Such equipment is often stored in a closed cabinet furnished with an electric light bulb or other heating unit. A cellulose acetate hood was constructed which is more economical and is readily accessible on the worker's desk or table. The hood was made airtight by cementing the edges with acetone; warping does not occur if small pieces of cellulose acetate are first dissolved in the acetone cement. A cylindrical shape was maintained by three wire hoops which are attached to the inside of the hood with adhesive tape. The bottom of the hood was placed in metallic mercury contained in a circular groove ¼ inch wide and ½ inch deep lathed in a wooden base. The mercury formed an airtight joint which is not sticky like the usual vaseline seals. Two concentric rings of soft rubber hose tacked to the inner and outer edges of the groove and in contact over its center also make an airtight joint when the bottom of the hood is slipped between them. This latter type of seal is preferable in laboratories where vaporized mercury may injure plants.

A dry atmosphere is maintained within the hood by placing calcium chloride in a container beneath a small wooden table which supports the instrument. Biological supply houses offer similar hoods which can be used provided they are sufficiently high to accommodate both the inner table and a precision instrument such as a microscope.

COFFEE

By Luis A. Gómez and José Lería Esmoris

Columnaris coffee variety superior in yield to Porto Rican variety.—Yields of the Columnaris variety of *Coffea arabica* L., from Java, and the West Indian variety were compared for the twelfth crop year in 1945. As a result of rather heavy pruning and rat damage, the Columnaris variety yielded only 606 pounds per acre of marketable coffee in 1945 against 675 pounds for the Porto Rican variety. The average acre-yields over a 12-year period, however, leave the Columnaris

variety well in the lead with 1,052 pounds as compared with the 630[9] pounds for the West Indian variety.

SOIL CONSERVATION

OBSERVATIONAL INVESTIGATIONS

By U. S. Allison,[10] Emery A. Telford,[10] Robley N. Jobe,[10] Norman F. Childers, and Edward P. Hume

Tropical kudzu gaining favor.—Although tropical kudzu, *Pueraria phaseoloides* (Roxb.) Benth., has been used for many years as a cover crop in rubber and cinchona plantations in the Dutch East Indies, its additional value as a soil erosion-control and forage crop in Puerto Rico has only recently been emphasized. An effort was made during the past year to increase trial plantings of tropical kudzu over the island as a whole. Several plantings were made for demonstration purposes at the agricultural substations sponsored by the Agricultural Extension Service. The Puerto Rico Agricultural Experiment Station at Río Piedras has a sizable planting of kudzu for experimental use with dairy cattle. In cooperation with Puerto Rican farmers over 175 acres were planted through the highland regions of the island.

The Federal station in cooperation with the Soil Conservation Service now has about 10 acres of kudzu either well established or newly planted, chiefly for experimental purposes to determine the fertilizer requirements, the possibility of growing it with various forage grasses, and the possibility of growing it between and on dwarf bucare stumps to increase forage and seed production per acre. Fertilizer experiments indicate that phosphorus is the element in Catalina soils most likely to be deficient. Inoculation of the seed with a special inoculum prepared for tropical kudzu by the Nitragin Co. in Milwaukee, Wisc., is recommended as one means of speeding up growth of the seedlings the first few months after planting.

From March to May 1946, about 1,200 tropical kudzu seedlings were grown in bamboo pots at the station and distributed to 4-H Club members in a contest to determine who could produce the most seed per plant during the following year. Prizes will be awarded to the top 10 members at the annual 4-H Club camp to be held in June 1947.

As a result of several newspaper and journal articles appearing on kudzu, requests were received for over 500 pounds of tropical kudzu seed from 20 foreign countries, the United States, and Puerto Rico. Over 400 pounds were distributed in Puerto Rico alone. The seed was collected from experimental plantings with labor furnished by the Insular Department of Agriculture and under the direction of the Soil Conservation Service.

Station land improved by soil-conservation practices.—With the aid of technicians provided by the Soil Conservation Service a soil and moisture conservation plan for a "bread-loaf" type field was completed on the newly acquired 22-acre Gómez property. More than a mile of broad-base 30-foot wide terraces were constructed with oxen team, plow, and by hand labor. These conservation practices make this land suitable for continuous cultivation.

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[9] This figure was in error in the 1945 annual report. The 11-year average yield for the Porto Rican variety should have read 652 instead of 668.
[10] United States Soil Conservation Service.

Over a mile of hillside ditches were constructed on land having a 40 to 55 percent slope. The ditches were laid out on grades and the vertical intervals were calculated to insure a minimum loss of soil and water. A strip of vegetative barrier was left above each ditch in order to filter any soil movement between ditches. One thousand feet of sod outlet channels were laid and temporarily pinned with bamboo strips to take care of runoff water. Some areas of the property are now well suited to the growing of vegetable crops while other sections are being prepared and used for a mango experimental orchard and for planting of bamboo and other plant introductions.

WEATHER

By William Vargas

Weather conditions during 1945-46 at Mayaguez similar to long-time averages.—The weather conditions at Mayaguez were more or less normal during the fiscal year 1945-46 (table 15). Total rainfall for the last 6 months of 1945 was 49.94 inches, which is close to the 47-year average of 50.01 inches. Total rainfall for the first semester of 1946 was 26.25 inches as compared with 30.34 inches for the 48-year average. The deficiency of about 4 inches of rainfall occurred during the relatively dry months of March, April, and May 1946.

The mean monthly temperature for the fiscal year 1945-46 was 77.0° F., which compared favorably with the 47-year average of 77.3°.

Table 15.—*Weather conditions at the Federal Experiment Station, Mayaguez, Puerto Rico, during the fiscal year 1945–46*

Month	Precipitation			Character of day			Temperature				
	Total[1]	Greatest in 24 hours	Days with 0.01 inch or more	Clear	Partly cloudy	Cloudy	Mean maximum	Mean minimum	Mean[2]	Maximum	Minimum
1945	*Inches*	*Inches*	*Number*	*Number*	*Number*	*Number*	°F.	°F.	°F.	°F.	°F.
July	9.86	3.25	20	5	12	13	89.0	69.1	79.1	92	67
August	10.52	3.50	18	7	12	12	89.4	68.1	78.8	92	65
September	14.15	3.12	23	4	9	17	86.3	69.7	78.0	93	67
October	8.32	1.96	18	11	14	6	89.8	68.4	79.1	93	65
November	4.51	1.30	12	11	12	7	87.1	66.0	76.0	91	60
December	2.58	1.05	7	17	8	6	86.9	65.1	76.0	90	62
1946											
January	2.56	0.82	9	13	12	6	85.4	65.0	75.2	89	62
February	3.18	1.25	7	8	9	11	85.5	62.6	74.0	89	60
March	2.20	1.03	7	15	14	2	86.3	63.0	74.7	91	59
April	2.99	0.80	13	14	9	7	87.2	64.5	75.9	90	62
May	6.04	1.59	21	4	8	19	88.1	66.9	77.5	93	61
June	9.28	2.61	16	3	13	14	89.5	67.9	78.7	93	65

[1]47-year average: July, 10.59 in.; August, 11.88 in.; September, 10.86 in.; October, 9.32 in.; November, 5.81 in.; and December, 2.56 in.
48-year average: January, 2.05 in.; February, 2.03 in.; March, 3.80 in.; April, 5.11 in.; May, 8.36 in.; and June, 8.88 in.
[2]46-year—Mean temperature: July, 79.0°; August, 79.3°; September, 79.4°; October, 79.2°; November, 77.6°; and December, 76.0°.
47-year—Mean temperature: January, 74.7°; February, 74.8°; March, 74.9°; April, 76.1°; May, 77.8°; and June, 78.7°.

PUBLICATIONS

DISTRIBUTION OF REPORTS

Station mailing list contained 1,060 names.—The annual report for the fiscal year 1944, 44 pages in length, was issued in June 1945; 311 copies were sent on request or exchange to individuals and institutions in the United States and possessions, and 417 to 43 foreign coun-

tries. The Spanish translation of the 1944 annual report was issued in February 1946, 242 copies of which were sent to interested individuals in Puerto Rico and 82 to Latin American countries.

During the year the current work of the station was published as usual in the quarterly mimeographed reports, totaling 131 pages, for interoffice circulation and for distribution on request to individuals professionally interested in the subjects covered. This report was circulated to 74 individuals, 15 of whom resided in 11 different foreign countries.

PUBLICATIONS ISSUED

The following bulletin was issued during the year:

Moore, Rufus H. Mineral deficiencies in *Derris elliptica.* Puerto Rico (Mayaguez) Fed. Expt. St. Bul. 43, 26 pp., illus. 1945

The following articles were published by the station staff in periodicals outside the Department:

Alvis, James K. Some advantages of using rubber tires on sugarcane carts in Puerto Rico. Rev. de Agr. de Puerto Rico 37: 73-76, illus. 1946.

Childers, Norman F., and Cibes, Héctor R. El cultivo de la vainilla en puerto rico. Rev. de Agr. de Puerto Rico 37: 3-14, illus. 1946.

Childers, Norman F., and Seguinot Robles, P. Bay rum in Puerto Rico. Agr. in Americas 5: 132-135. 1945.

Childers, Norman F., and Seguinot Robles, P. La industria de la malagueta en puerto rico. Rev. de Agr. de Puerto Rico 36: 56-59, illus. 1945.

Harper, Roy E. USDA-34—A tropical sweet corn. Agr. in Americas 6: 74-75, illus. 1946.

Harper, Roy E., and Winters, Harold F. Cinchona investigations in Puerto Rico. Agr. in Americas 6: 3-32, illus. 1946.

Jones, Merriam A., and Arrillaga, Noemí G. An apparatus for columnar absorp-Anal. 34: 92, illus. 1945.

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